DALHOUSIE UNIVERSITY

BIOSAFETY MANUAL

2015

Dalhousie University Biosafety Manual

All new staff and students are required to read this document prior to beginning any work using biological agents. After reading please date and sign to acknowledge that you have done so.

Name (please print)	Signature	Date

Telephone Contact Information

1. Incidents Involving Biohazardous Materials

Biological Safety Officer	Pam Gallant	902-494-4452

2. Emergency Numbers

EMERGENCIES	**902-494-4109**
Dalhousie Security	902-494-6400
Dalhousie Health Services	902-494-2171
Dalhousie Maintenance	902-494-3345/902-494-6400 (after hours)

3. Members of the Biosafety Committee (2015)

Halifax:	
Dr. R. Anderson	902-494-1063
Dr. S. Craig	902-494-1270
Dr. T. Hatchette	902-473-6885
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Table of Contents

Chapter 1: Introduction

Dalhousie University is committed to providing a safe laboratory environment for its faculty, staff, students, and visitors. Dalhousie University's Biosafety Manual and Policies are designed to help foster a safe and healthy environment that adequately supports work and study. The goal of the Biosafety Manual is to minimize the risk of laboratory incidents by providing laboratory personnel with the proper information needed to work safely. The Biosafety Manual also intends to aid in the protection of laboratory personnel and the surrounding environment from possible exposure to biohazardous material.

The Biosafety Manual provides information, guidelines, and policies that should be used in conjunction with other resources to safely work with and in the environment of biohazardous materials.

Workers must also be aware of the complex web of laws and regulations that place special responsibilities on people, specifically:

Canada:
Hazardous Products Act, 1985
Health of Animals Act, 1990
Transportation of Dangerous Goods Act, 1992
Controlled Drugs and Substances Act, 1996
Nuclear Safety and Control Act, 1997
Canadian Environmental Protection Act, 1999
Human Pathogens and Toxins Act, 2009

Nova Scotia:
Environment Act, 1994-95
Occupational Health and Safety Act, 1996

Halifax Regional Municipality By-Laws:
Solid Waste Collection and Disposal (By-law S-600 as amended), 1999
Wastewater Discharge (By-law W-101), 2001

1.1 Definitions

Biological material – material present or produced in a living organism that contains genetic information and is capable of reproducing or being reproduced in a biological system. This material may pose a risk to health and safety, or the environment.

Biohazard – biological material or a condition that constitutes a hazard to the health and safety of humans, animals, or the environment.

Biosafety – practices, procedures, and uses of equipment to ensure safe conditions when working with potentially infectious microorganisms and other biohazardous materials. Biosafety is intended to provide prevention of risk to human health and safety, and environmental exposure from disease or harmful biological agents.

1.2 Responsibilities

The Biological Safety Officer, Principal Investigators, and laboratory personnel must work together to ensure safety when working with biohazardous materials. The day-to-day operation of the Biosafety Program is overseen by the Biological Safety Officer. Principal Investigators are primarily responsible for the safe operations of their laboratory and must ensure safe work practices are implemented, while laboratory personnel must follow the procedures as outlined in this manual and by their supervisor. Additional responsibilities are outlined as follows:

Biological Safety Officer:

- Develop procedures for the implementation of biosafety guidelines and policies to ensure compliance with the regulations for use and exposure to biohazardous materials
- Perform visits, inspections, and audits to ensure compliance
- Consult with Principal Investigators to ensure the appropriate containment levels are established
- Provide support, advice, and consultation on any biosafety issue
- Provide training for persons using biohazardous materials
- Investigate all incidents relating to laboratory biosafety

Principal Investigator (PI):

- Obtain and renew biosafety permits
- Participate in biosafety training and any other relevant training programs
- Ensure all new laboratory personnel have read the Biosafety Manual and receive the proper training and instruction in order to work safely with potentially biohazardous material
- Ensure the safe work practices outlined in the Biosafety Manual and Policies are met and followed by all laboratory personnel
- Ensure all personnel are provided with and wear the appropriate personal protective equipment
- Regularly inspect their area of supervision for hazardous conditions
- Ensure all laboratory equipment functions properly and the inspection/certification is current
- Ensure accessible and accurate inventory records are maintained
- Investigate any laboratory personnel security and safety concerns
- Ensure all incidents are reported and investigated

Laboratory Personnel:

- Follow the policies and safe work practices outlined in the Biosafety Manual or by their supervisor

- Participate in all training courses as directed by their supervisor
- Use the appropriate personal protective equipment when working with biohazardous material
- Ensure full understanding of the risks associated with the biohazards used in the laboratory activities and seek information when unsure about any potential biohazard
- Report all incidents, laboratory acquired infections, and unsafe conditions to the laboratory supervisor immediately

Chapter 2: Regulatory Agencies

2.1 Canadian Food Inspection Agency

The Canadian Food Inspection Agency (CFIA) works with Agency scientists and technical experts to establish the biocontainment levels, procedures, and protocols that are needed to work safely with animal and zoonotic pathogens, chemical hazards, and plant pests of quarantine significance, and to protect laboratory staff, the Canadian public, and the environment.

In accordance with the Health of Animals Act and its regulations, the CFIA is responsible for issuing permits for non-indigenous animal pathogens, emerging animal disease pathogens, aquatic and plant pathogens, as well as animals, animal products and by-products, tissue, sera, and blood that are infected with animal pathogens.

In addition to the Animal Pathogen Import Program as described above, information the CFIA provides includes the containment standards for Facilities Handling Aquatic Animal Pathogens, Foreign Animal Disease Diagnostic Laboratories, and Facilities Handling Plant Pests.

The CFIA also provides a framework for the oversight of Laboratory Management for facilities conducting testing under the CFIA's mandate.

As of April 1, 2013, some programs at the CFIA's Office of Biohazard Containment and Safety were transferred to the Public Health Agency of Canada (PHAC) (refer to Section 2.2).

The CFIA and PHAC have joined forces to update and synchronize three existing Canadian biosafety standards and guidelines by creating the *Canadian Biosafety Standards and Guidelines* (CBSG). The CBSG has replaced the *Laboratory Biosafety Guidelines,* the *Containment Standards for Veterinary Facilities* and *Containment Standards for Laboratories, Animal Facilities and Post Mortem Rooms Handling Prion Disease Agents.* Further information on the CBSG can be found in Section 2.2.

2.2 Public Health Agency of Canada

To strengthen its ability to promote and protect the health and safety of Canadians, in 2004 the Government of Canada established the Public Health Agency of Canada (PHAC). PHAC was confirmed as a legal entity in 2006 by the Public Health Agency of Canada Act. The creation of PHAC marked the beginning of a new approach to federal leadership and collaboration with provinces and territories on public health. It responds to a consensus from the provinces, public health experts and concerned citizens on the need for federal leadership on public health to be consolidated in a public agency.

PHAC serves as the national authority for the biosafety and biosecurity of human pathogens and toxins in Canada. The Agency's Center for Biosecurity administers and

enforces the Human Pathogens Importation Regulations (HPIR) and the Human Pathogens and Toxins Act (HPTA). The HPTA will be supported by the Human Pathogens and Toxins Regulations (HPTR) when they come into force on December 31, 2015, at which time the HPIR will be repealed.

PHAC is responsible for issuing import permits for human and terrestrial animal pathogens, with the exception of non-indigenous animal pathogens and pathogens causing emerging animal diseases. PHAC is also responsible for issuing Containment Level 2 compliance letters and the certification of High Biocontainment laboratories.

PHAC provides tools to individuals who design, operate or work in laboratories in which human pathogens are manipulated for diagnostic, research or development purposes. These laboratories may be located in universities, hospitals, government departments or industrial settings. Tools provided by PHAC include Pathogen Safety Data Sheets (PSDS) for infectious substances, which provide detailed descriptions of the hazardous properties of specific human pathogens and toxins.

Also available from PHAC are the *Canadian Biosafety Standards and Guidelines* (CBSG). Developed jointly by PHAC and CFIA, the CBSG is a technical document which provides details on the physical containment and operational practice requirements for facilities possessing, handling, storing, or using human and terrestrial animal pathogens or toxins. Following the standards and guidelines outlined in this document will help protect personnel from exposure to infectious materials and toxins, as well as aid in the prevention of the inadvertent release of these materials. The CBSG is also used by PHAC and CFIA to verify regulatory compliance of facilities where infectious materials or toxins are handled and stored, as well as for the certification or recertification of containment zones.

Chapter 3: Program Organization & Administration

The policies, regulations, and procedures of the Biosafety Program shall apply to all activities involving the use, storage, transportation, and disposal of biological agents (including toxins) in or on the buildings and grounds of Dalhousie University and in off-site locations occupied by Dalhousie faculty, staff, and students.

The organization of the Biosafety Program include the following:

- Biosafety Committee
- Biological Safety Officer
- Project Directors (Principal Investigators)
- Users of biological agents

3.1 Biosafety Committee

Policy Statement
The Biological Safety Officer is responsible for the day-to-day operations of the Biosafety Program. He/she has the authority to implement and enforce the biosafety program encompassing the use, handling, storage, and disposal of biohazardous materials in accordance with regulatory requirements of the Public Health Agency of Canada and the Canadian Food Inspection Agency.

The Biosafety Committee is appointed by and accountable to the VP, Finance.

All faculty, staff, and students are expected to take individual responsibility for safe work practices and procedures so as to safeguard their own individual health and well-being as well as that of their colleagues.

Committee Membership
The Committee shall consist of a minimum of eight members drawn from those engaged in work involving the use of biological agents. Every effort should be made to ensure that all faculties in which biological agents are in use are represented. In addition the following shall be members:

- Biological Safety Officer
- Director of Environmental Health & Safety
- University veterinarian
- Security representative
- Facilities Management representative
- Dental Clinic representative

Terms of Reference

1. Establish and regularly review policies and procedures for the safe use of biohazardous materials.
2. Assist the Biological Safety Officer, where necessary, with the preparation and submission of reports to regulatory agencies.
3. Establish and review worker training programs on an annual basis.
4. Advise the Biological Safety Officer, if necessary, on the processing of internal permit applications and approval of space and facilities to be used for projects involving those items listed in item (1).
5. Receive reports of any incidents or accidents involving biohazardous materials, arrange for investigations where warranted, and assist the Biological Safety Officer with the required reporting to appropriate bodies.
6. Advise senior management of the need for additional resources to improve the Biosafety Program
7. Conduct an annual audit of the Biosafety Program.
8. Maintain written records of meetings, actions, incidents, and unusual occurrences along with recommendations.

Reporting Structure

The Biosafety Committee is accountable to the VP, Finance. Dalhousie University's Environmental Health and Safety Committee shall be advised of the Biosafety Committee's proceedings, and in turn, may refer matters to the Biosafety Committee for consideration or action.

Membership Term

Committee members will normally be appointed for a three year term. Members may be reappointed to serve subsequent terms.

Chairperson

The Chairperson shall serve a three year term. The Chairperson will be elected by the voting members.

Meetings

The Biosafety Committee shall meet four times yearly – typically September, December, March, and June. Special meetings, however, may be called at any time. The schedule for the year will be established in September.

Agenda

Any member may place items on the agenda for discussion. Items for inclusion on the agenda should be received by the Environmental Health and Safety Office at least one week prior to the scheduled meeting to allow time for distribution of relevant documents to committee members.

Conduct of Meeting

Meetings will be conducted by the Chairperson. In the absence of the Chairperson, voting members in attendance will select a member as acting Chairperson.

Quorum

At all meetings, a quorum will be one half of the membership.

Voting

The Committee will normally seek to operate by consensus without the need of formal votes. When a member requests a formal vote, a motion will be carried when supported by a simple majority of members.

Chapter 4: Laboratory Biosafety

Safe laboratory practice is critical in preventing exposure when working with biohazardous materials. Anyone planning to work with biohazardous materials must be trained prior to beginning work. Supervisors are responsible for ensuring that all personnel in their laboratories are adequately trained.

4.1 Biosafety Training

Prior to any work with biohazardous materials, a new worker must read both the Dalhousie University Biosafety Manual as well as the Public Health Agency of Canada's *Canadian Biosafety Standards and Guidelines*, and sign acknowledgment of having done so. As well, the Principal Investigators/Supervisors are responsible for training their workers in all laboratory specific procedures as defined in the Standard Operating Procedures (SOP's). All new workers are required to take Dalhousie University's Biosafety Training course. Workers must take refresher training every 4 years. Topics that may be covered in any Biosafety training session might include:

- Access/security controls
- Use of safety equipment
- Health hazards
- Safe work procedures
- Emergency procedures

4.2 Access/Security Controls

The *Canadian Biosafety Standards and Guidelines* requires the international biohazard warning symbol (Figure 1) to be displayed if biohazardous materials, including body fluids, unfixed cell, tissue or organ cultures, viral, bacterial, fungal or parasitic agents requiring BSL 2 or greater are present. The Containment Level of the laboratory must also be indicated. Laboratory doors should be locked when the laboratory is unoccupied and only authorized persons are permitted to enter laboratory working areas. Principal Investigators should also maintain a current approved worker list with the Biological Safety Officer. Children under the age of 14 years are not to be permitted to enter laboratory working areas.

Figure 1. Universal biohazard symbol.

4.3 Use of Personal Protective Equipment (PPE)

A properly fastened laboratory coat, enclosed footwear with no or low heels, and gloves need to be worn in any microbiology laboratory. Laboratory coats, enclosed footwear, and gloves prevent biohazardous materials from contact with the skin, including any areas where there might be cuts, abrasions, or dermatitis. The legs are a vulnerable area if uncovered, so it is inappropriate to wear short skirts or shorts. A properly fastened ***laboratory coat*** also protects street clothing from becoming contaminated and prevents possible cross contamination from any normal flora present on the skin. It is important that laboratory coats remain in the laboratory to prevent spread of contamination to non-laboratory areas or your home.

Enclosed footwear with no or low heels protect the feet from spills as well as injuries from dropped sharps.

Appropriate ***gloves*** need to be worn for all procedures that may involve direct or accidental contact with biohazardous materials. Latex or nitrile gloves offer a high level of dexterity and a higher level of sensitivity, however, they don't offer a great deal of protection from needle sticks, animal bites, or sharps. Double gloving is recommended. Compatibility with chemicals being handled should also be considered. Glove fabrics differ in their resistance to permeation by different chemicals. Gloves should over wrap the cuff and lower sleeve of your laboratory coat. ***Open cuts or wounds must be covered before applying gloves. Laboratory activity should be denied to persons with a weeping dermatitis.***

In addition to the minimal protective equipment described above, other protective equipment might be needed when working with infectious agents.

Safety glasses or ***face shields*** offer protection from splashes of biohazardous materials, impacting objects and ultraviolet light sources.

Chapter 9 of the *Canadian Biosafety Standards and Guidelines* details the use of personal protective equipment in Containment Level 2 laboratories.

4.4 Safe Work Procedures

In accordance with the Canadian Biosafety Standards and Guidelines and Dalhousie University's Biosafety Policies:

1. The laboratory should be kept neat, clean, and orderly.
2. Laboratory access should be limited to approved workers to avoid unnecessary exposure to ancillary personnel and to maintain the security of biological agents.
3. All laboratory personnel must become familiar with the biohazards that are likely to be encountered in their particular laboratories prior to the start of any work with biohazardous agents. A procedural manual should be available for all staff.
4. Personnel must receive training on the potential hazards associated with the work involved and the necessary precautions to prevent exposure to any uncontrolled release of the biological agent. Training must be documented.
5. Laboratory workers should be protected by immunization where appropriate.
6. Appropriate PPE must be available and worn. PPE is not be worn outside the laboratory areas. Spare lab coats should be made available to visitors to the laboratory. Appropriate PPE includes, but is not limited to – laboratory coats/gowns, disposable gloves, masks, and safety glasses.
7. Eating, drinking, storing of food, or other personal materials is not permitted in the laboratory.
8. The wearing of jewellery in the laboratory is discouraged and long hair is required to be tied back.
9. Applying cosmetics and handling contact lenses should not be permitted. The wearing of contact lenses is only permitted when other forms of eyewear are not acceptable. In these cases, when hazardous operations are performed safety glasses should be worn as well.
10. Hands must be washed after gloves have been removed using proper hand washing technique (Figure 3).
11. Avoid forcefully aspirating or expelling liquids.
12. When pipetting is involved in laboratory work, workers must use pipettors and pipette aids. ***Mouth pipetting is strictly forbidden***.
13. The use of needles, syringes, and other sharp objects should be strictly limited to avoid accidental inoculation. Contaminated needles should not be bent, sheared, recapped, or removed from the syringe. These must be promptly placed in an approved sharps container. ***Procedures that require recapping of needles is to be discouraged****.*
14. Work surfaces must be decontaminated with a suitable disinfectant at the end of the day and after any spill of potentially biohazardous material. Spills must be reported to the Environmental Health & Safety Office.
15. To avoid accidental spills or leaks, secondary containment should be incorporated when transporting biological agents. Shipping of biological agents to another facility should be overseen by an individual who has the appropriate Transportation of Dangerous Goods (TDG) training. If in doubt, contact the Environmental Health & Safety Office.

Glove Removal

To avoid contaminating your hands when removing gloves, care must be taken to avoid touching the skin with the outside of the glove. The following technique for glove removal should be employed (Figure 2):

1. With both hands gloved, peel one glove off from the top to bottom and hold it with the gloved hand.
2. Grasp the inside of the cuff of the second glove with the exposed hand and peel it off from the top, tucking the first glove inside the second.
3. Dispose of the gloves promptly to an appropriate waste container.
4. ***Never touch the outside of the glove with bare skin***.
5. Wash hands as soon as possible using proper hand washing technique.

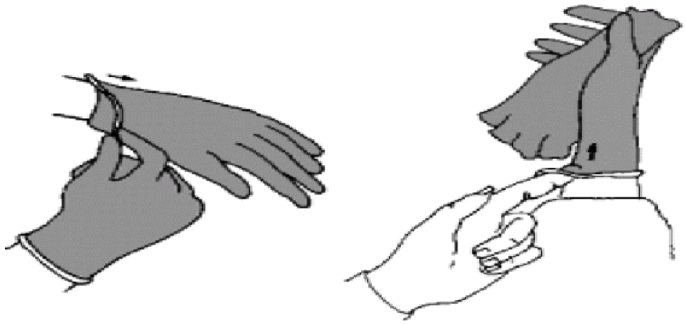


Figure 2. Proper glove removal technique.

Hand Washing

Hands should be washed with soap under clean running water for a minimum of 15 seconds before leaving the working area (Figure 3). Using hot water and soap, the hands should be rubbed together to create a lather. The hands should be thoroughly scrubbed, including wrists, between fingers, under fingernails, and the backs of the hands. Soap should be rinsed off thoroughly. Using paper towel, hands should be dried completely. Paper towel should be used to turn off the faucet and open the door. Hand sanitizers may be used if they are effective against the pathogen or toxin used.

Figure 3. Proper hand washing technique.

[1] Retrieved from: http://www.simcoemuskokahealth.org/Libraries/DRAW_InfectiousDisease/Handwashing.sflb.ashx

4.5 Containment Level 2 Requirements

In addition to the general requirements listed above, the following list describes minimum operational procedures for Containment Level 2 laboratories.

1. The universal biohazard symbol must be posted on laboratory doors. In addition to the universal biohazard symbol (Figure 1), the containment level of the laboratory must be posted at points of entry.
2. Non-university visitors to the laboratory are required to sign in upon entering the laboratory. Laboratory coats should be provided to these visitors.
3. Biosafety Cabinets (BSCs) must be used for procedures that may produce aerosols or that involve work with high concentrations and/or volumes of the agent. Animal handling and necropsy should be performed in the BSC as well.
4. Biological agents that appear on either The Australia Group's Human and Animal Pathogens and Toxins export control list or the Centers for Disease Control and Prevention (CDC) Select Agents and Toxins list should be stored in a locked area when an authorized worker is not present in the laboratory.

4.6 Potentially Hazardous Operations and Preventive Measures to Reduce Risks

In the laboratory environment, there are many hazardous procedures that have the potential to cause accidental injection and/or create aerosols. Safe practices can prevent accidental injection, and limit the formation and dispersal of aerosols. A summary of equipment related hazards is found in Table 1.

Table 1. Equipment related hazards and methods that can be employed to reduce or eliminate the hazard.

Equipment	Hazard	How to Reduce/Eliminate Hazard
Needles and Syringes	Accidental needle stick, aerosol, spills	- Do not recap needles - Use a luer lock syringe - Minimize air bubbles and frothing on filling - Avoid mixing infectious liquids - Wrap the needle and stopper with cotton pad moistened with disinfectant prior to withdrawing needle from septum - Expel air bubbles into a cotton pad moistened with a disinfectant
Centrifuges	Aerosols, splashing, broken tubes	- Use sealable buckets or rotors - Load and unload buckets or rotors in a BSC - Wait 10 minutes for aerosols to settle before opening cemtrifuge lid
Ultracentrifuges	Aerosols, splashing, broken tubes	- Install a HEPA filter between centrifuge and vacuum pump - Load and unload buckets or rotors in a BSC - Maintain a preventative maintenance program to reduce the risk of mechanical failure
Anaerobic jars	Explosion	- Ensure integrity of wire capsule around catalyst
Desiccators	Implosion, dispersing glass fragments and infectious materials	- Double contain unit
Homogenizer, tissue grinder	Aerosols, leakage and container breakage	- Operate in a BSC - Wait 30 minutes before opening blender bowl to allow the aerosol to settle - If manual tissue grinders are used, hold tube in a wad of absorbent material
Sonicators, ultrasonic cleaners	Aerosols, noise, dermatitis	- Operate and open units in a BSC or sealed unit (only if equipment does not interrupt airflow) - Ensure insulation to protect against subharmonics - Wear gloves to protect against high proficiency plus detergent action on skin
Culture stirrers, shakers, agitators	Aerosols, splashing and spillage	- Operate in a BSC or specially designed primary containment - Use heavy duty screw-capped culture flasks
Lyophilizers	Aerosols, direct contact contamination	- Use O-ring connectors to seal the unit - Use air filters to protect vacuum lines - Use an appropriate method of decontamination - Provide an all metal moisture trap and vapour condenser - Use only glassware designed for vacuum work
Water baths	Growth of microorganisms	- Ensure regular cleaning and disinfection - Autoclave

Needles, Syringes, and Other Sharps
The greatest risk of using sharps is accidental injection and the creation of aerosols.

1. Needles and syringes should only be used when there is no reasonable alternative to handle contaminated liquids.
2. Sharps should never be bent, sheared, recapped, nor have needles removed from syringes. If a needle must be recapped do so by using forceps or using single-handed scooping (Figure 5).
3. Avoid the production of air bubbles when filling a syringe. Expel any trapped air into a pad moistened with disinfectant placed over the needle tip.
4. Maintain an approved sharps container in the immediate work area. Never over-fill these containers beyond ⅔ capacity.

Preventing Needle Stick Injuries
Needle stick injuries are wounds caused by needles that accidentally puncture the skin. These injuries can occur at any time when needles are being used, disassembled, or being disposed of.

Needle stick injuries can transmit infectious diseases, especially blood-borne viruses.

Work practices that increase the risk of needle stick injury
- Recapping needles
- Transferring infectious material between containers
- Failure to dispose of used needles in puncture-resistant sharps containers

How can needle stick injuries be prevented?
Employee training – Workers need to know how to safely use and dispose of sharps, as well as understand the risk associated with needle stick injuries, and know how to prevent them.

Safety-engineered syringes – Safety-engineered syringes are recommended as they have safety features built into the product. Safety-engineered syringes include features such as protective shields, retractable needles, and blunt tips (Figure 4).

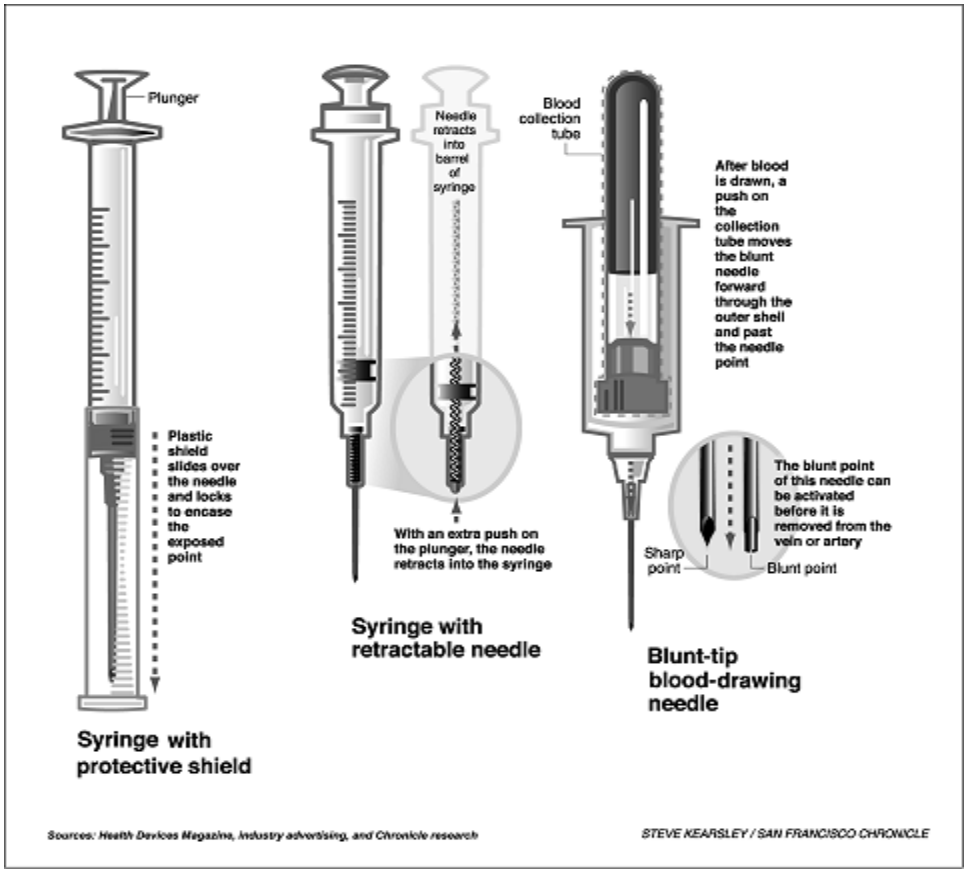


Figure 4. Examples of safety-engineered syringes.

Safe recapping procedures

In situations where recapping is necessary, two techniques may be employed.

1. *Single handed scooping* - The risks of recapping can be reduced if the cap is laid on a flat surface and scooped onto the tip of the syringe held in one hand. The free hand should be kept away from the sheath and well behind the exposed needle (Figure 5).

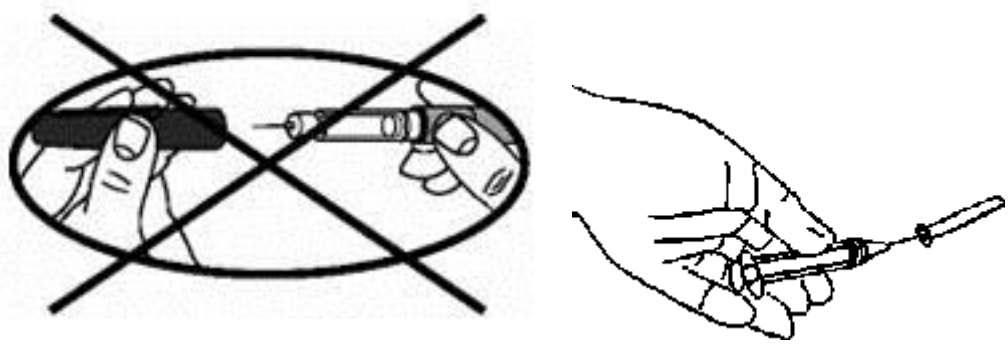


Figure 5. Single handed scooping technique to recap a needle.

2. *Recapping Devices* - Several devices are available for recapping needles safely. Some devices permit single-handed recapping by parking a needle cap on a flat surface (Figure 6). Other devices are designed to protect the hand that holds the cap during two-handed recapping procedures.

Figure 6. Example of a recapping device.

Disposal

Disposal containers for sharps must be readily available. These sharps containers must be puncture proof. Disposal must follow university disposal procedures, please refer to Dalhousie University's Policy for Management of Biological Waste.

Reporting

Many incidents go unreported. Reporting of needle stick and other sharps injuries (Section 8.5 Incident Reporting) assists the Biosafety Office with determining the rate of needle stick injuries, investigating the cause of such injuries, ensuring that workers receive proper treatment, identifying areas where the prevention program needs improvement and providing practical strategies for dealing with the problem.

Pipetting

The greatest hazard of pipetting is the potential for the creation of aerosols and splashing.

1. Mouth pipetting is prohibited, mechanical pipetting aids must be used.
2. Where possible pipette biohazardous materials in a biosafety cabinet.
3. Never discharge biohazardous materials forcibly from pipettes. "To deliver" pipettes are recommended.
4. To avoid splashing, biohazardous material should be dispensed from the pipette by allowing it to run down the wall of the receiving container.
5. After use, re-usable pipettes should be placed in a proper container of germicidal liquid to completely cover them. The germicidal liquid must be effective against the pathogen in use.

Centrifugation
The greatest risk of centrifugation is the potential for the creation of aerosols.

1. Sealed tubes and safety buckets that seal with O-rings should be used. Prior to use O-rings should be inspected for damage to avoid the possibility of spills.
2. To avoid leaks, do not over fill centrifuge tubes. Wipe the outside of the tube with a disinfectant once filled and sealed prior to loading into the centrifuge.
3. Safety buckets should be removed from the centrifuge and loaded/unloaded in the BSC.
4. If “biosafety buckets” are not available, wait a minimum of 10 minutes after the spin is complete before opening the centrifuge.

Blending, Grinding, Sonicating, Lyophilizing
The greatest risk when performing any of these operations is the potential for aerosol production.

1. Perform these operations in a biosafety cabinet.
2. Use safety blenders which are designed to prevent leakage from the bottom of the blender jar and which can withstand autoclaving.
3. Avoid the use of glass blender jars.
4. Place a towel moistened with a disinfectant over the top of the blender while it is in operation. This practice can be adapted for sonicators and grinders as well.
5. Allow aerosols to settle for at least thirty minutes before opening blenders, grinders or sonicators.
6. Filter lyophilizer vacuum pump exhaust through HEPA (high efficiency particulate air) filters or vent to a biosafety cabinet.
7. Use polypropylene tubes in place of glass for storing biohazardous materials in liquid nitrogen.

Chapter 5: Biological Material

5.1 General Classifications

Biohazardous materials are those materials of a biological origin that could cause harm to humans, domestic or wild animals, or plants. The risk can be direct through infection or indirect through damage to the environment. Biohazardous agents can be classified into the following groups:

- Microorganisms
- Parasites
- Toxins
- Prions
- Recombinant DNA
- Animals
- Viral Vectors

Microorganisms **-** This group includes bacteria, viruses, fungi, and protozoa.

Parasites **-** This group refers to multi-cellular organisms that can only survive if they live within a host.

Toxins **-** Toxins are poisonous substances that are produced by bacteria, animals, or plants. They are usually active at a very low concentration. They vary greatly in their severity, ranging from mildly poisonous to deadly.

5.2 Prions

Some progressive neurological diseases (e.g., spongiform encephalopathies) are caused by proteins referred to as prions. Examples of such disease are:

- Creutzfeldt-Jakob disease in humans
- Mad Cow Disease in bovines
- Scrapie in sheep and goats

Prions are resistant to destruction by chemical and physical procedures that normally inactivate viruses, including autoclave sterilization.

The following precautions should be observed when handling neurological tissue from infected or potentially infected humans or animals:

- Handle as Risk Group 2 or higher (see Chapter 6 for a description of Risk Groups)
- Handle formalin fixed tissues and paraffin-embedded blocks as infectious
- Follow specific pertinent up-to-date disinfection protocols for these pathogens

5.3 Recombinant DNA

Molecules are constructed outside living cells by joining natural or synthetic DNA segments in such a way that they can replicate in a living cell. Recombinant DNA usually involves putting a gene from one organism into the genome of a different organism, generally of a different species. A gene in its own natural genome may not pose a risk, however, the risk level can change when the gene is present in a foreign genome or when it is modified in some way that affects its expression or function.

The *Canadian Biosafety Standards and* Guidelines indicates that in evaluating the level of risk for manipulations, the following should be considered:

- Gene(s) being transferred
- Modification to genes already present in the organism
- Gene expression in the recombinant organism
- Biological containment offered by the host organism
- Interactions between the gene(s) transferred and the host vector systems
- The viability of the host vector systems

Please also refer to a comprehensive document produced by NIH entitled NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines).

5.4 Animals

Animals that are used in a research setting or those found in the field can pose a number of different risks, including physical injury from bites, scratches, or kicks. Exposure to animal dander can result in allergies or other adverse reactions. Animals can also harbor agents that cause zoonotic diseases.

By definition, all work involving animals is considered to be a biohazard risk since animals can harbour infectious organisms that can be transmitted to humans.

Dalhousie University Animal Care Protocols must be followed.

Animal Cells, Blood and Body Fluids, and Fixed Tissue

The biological hazards of animal cells, tissues, blood, and body fluids arise from the possibility that they might transmit infectious agents. It is prudent to consider all cell lines to be potentially infectious. Cell lines known or suspected to contain agents, or primary cultures from animals and humans known or suspected to be infected, should be assigned to the risk group for the suspected agent.

Primate cell lines, primate tissues, cell lines exposed to or transformed by primate oncogenic virus, samples of human tissue and fluids, mycoplasma-containing cell lines, and cell lines new to the laboratory should be handled at Containment Level 2. While

handling items such as human blood and body fluids, workers must be aware that they may contain pathogens such as influenza, HIV, and hepatitis.

All anatomical specimens should be handled at Containment Level 2 because of the possibility of encountering such viruses as Hepatitis B or the possibility of encountering prions.

Factors such as the particular source of the material, the volume and concentration of the agent, the extent of culturing and incubation, the types of manipulations to be conducted, and the use of additional precautions could influence the containment level that is required.

All other animal tissue culture work can be handled at Containment Level 1.

Animal Necropsy

Necropsy is the term used for an animal post mortem examination. The procedures listed below outline general biosafety considerations for necropsy of animals known to be harboring infectious agents. These are general procedures only, more specific project related procedures may be required.

Containment Considerations:

1. Necropsies on animals must be conducted at a BSL 2/ABSL 2 level. Higher level BSL 2+ practices may be required if infection with zoonotic agents is known or suspected.
2. Necropsy of small animals must be conducted in a biosafety cabinet.
3. If a large animal is to be necropsied, consult with your facility veterinarian.
4. Unessential personnel should not be present during the procedure.

Personal Protective Equipment (PPE) Considerations:

1. A laboratory coat is acceptable if the necropsy is performed in a biosafety cabinet. Rear closing gowns must be worn if the procedure is performed outside the BSC. Non-disposable PPE should be autoclaved before laundering.
2. Impervious gloves must be worn. Double gloving is recommended with the outer pair being changed if there is any evidence of damage to the glove. Consider cut-resistant gloves when using scalpels or other sharp instruments.
3. Safety goggles with side shields should be worn. If there is potential for splashing a face shield should be worn.
4. If aerosols may be generated, particularly if the procedure is performed outside the BSC, properly fitted respirators should be worn.
5. Shoe covers should be worn if performing necropsy outside the BSC.
6. Doff your PPE prior to entering clean areas.

Procedural Considerations:

1. Specimens should be handled and treated with the assumption that zoonotic disease is present. Specimens should be placed in leak-proof containers. The outside of the containers should be disinfected prior to leaving the necropsy area.
2. Tools and instruments should be used in such a way as to minimize “stick and cut” hazards.
3. Hand tools are preferred to power tools, as power tools can generate aerosols.
4. Disposable sharps, needles, blades, glass slides, etc. must be discarded into an appropriate sharps container for decontamination.

Disinfection Considerations:

1. Necropsy tools must be cleaned to remove gross contamination, then autoclaved or disinfected immediately after use. The disinfectant must be suitable for the hazard present and sufficient contact time must be allowed.
2. The BSC, necropsy table, and all other work surfaces must be thoroughly disinfected and cleaned at the completion of the procedure.
3. Avoid high pressure wash until after the disinfectant has remained in contact with the surfaces for the prescribed time.
4. Carcasses must be disposed of as per university policies.
5. Always wash hands thoroughly after removing PPE and before leaving the necropsy area.

5.5 Viral Vectors

There is inherent risk when working with viral vectors. The following information applies to work with adenoviral vectors, adeno-associated viral vectors, lentiviral vectors, and retroviral vectors (Table 2).

Viral vectors are often designed to enter human cells and deliver genes of interest. Viral vectors are usually replication-deficient. There are several biosafety concerns that may arise, specifically:

1. Tropism (host range) – viral vectors that can enter human cells are often used.
2. Replication-deficient viral vectors can gain back the deleted genes required for replication through recombination.
3. Genes can be expressed in tissues and/or organisms where they are not normally expressed.

Work with these agents should be performed at BSL 2. Containment level 2 practices must be rigorously followed. Some BSL 3 practices may also have to be followed. This is referred to BSL 2+.

Table 2. Level of containment, additional precautions and effective disinfection agents required for working with select viral vectors.

Viral Vector	Biosafety Level	Animal Biosafety Level	Hazard	Additional Precautions	Disinfection
Adenovirus	2	2 (up to 5 days post injection)	Droplets, aerosol, injection	Work within BSC PPE to include gowns and gloves	10% bleach (recommended); 2% gluteraldehyde
Adeno-associated virus	2	2 (72 hours post injection)	Droplets, aerosol, injection	PPE	10% bleach
Lentivirus Retrovirus	2	2 (7 days post injection, or longer as required)	Injection, splash to face	Work within BSC PPE to include gowns, gloves, eye protection	10% bleach (recommended); 2% glutaralehyde; 4% formaldehyde; 70% ethanol

The laboratory must meet the requirements of the *Canadian Biosafety Standards and Guidelines*. Please also refer to the NIH document Biosafety Considerations for Research with Lentiviral Vectors.

Laboratory Practices for Working with Viral Vectors

1. Laboratory doors must remain closed.
2. Work with viral vectors must be performed in a biosafety cabinet.
3. For aspiration, use a plastic vacuum flask with a second vacuum flask connected to it as a backup, with non-collapsible tubing capable of withstanding disinfection. (Figure 7).
4. Standard work procedures as outlined in “Effective Use of BSCs” (Section 7.4) must be followed.
5. Viral vector stocks should be tested for the presence of replication competent virus prior to introduction into research animals.
6. Animals must be handled at ABSL 2 conditions.
7. Take care not to create aerosols when emptying animal bedding, cleaning cages, and rooms.
8. All necropsy must be performed in a containment cabinet under BSL 2/ABSL 2 conditions.
9. Note that animals infected with adenoviral vectors may shed the virus for up to 72 hours.

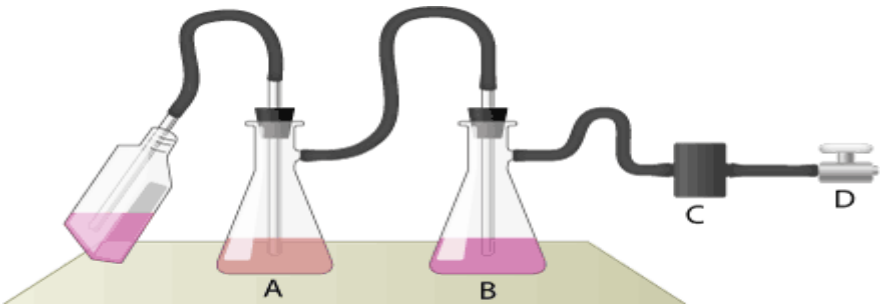


Figure 7. Example of correct vacuum system set-up to prevent internal contamination (A: collects liquid; B: minimizes splatters; C: in-line HEPA filter; D: vacuum line).

5.6 Bloodborne Pathogens (BBP)

Bloodborne pathogens are microorganisms that are transmitted via human blood and that have the potential to cause illness in individuals who may be exposed to them. The pathogens of greatest concern in the workplace are:

- Hepatitis B virus (HBV)
- Hepatitis C virus (HCV)
- Human Immunodeficiency Virus (HIV)

These organisms may be present in:

- Blood (any component)
- Unfixed human tissue or organs
- Experimental (contaminated) cell cultures (human or animal)
- Semen
- Vaginal secretions
- Cerebrospinal fluid
- Synovial fluid
- Pleural fluid
- Pericardial fluid
- Peritoneal fluid
- Amniotic fluid
- Saliva
- Any other bodily fluid visibly contaminated with blood

More information on the BBP listed above can be found in Table 3.

Table 3. Method of transmission and available infection control for bloodborne pathogens of concern in the workplace.

Disease	Method of Transmission	Infection Control
Hepatitis B – an infection of the liver caused by HBV. Symptoms include fatigue, loss of appetite, abdominal pain, vomiting, jaundice	-Contact with blood, body fluids and/or unfixed tissues	-Standard precautions -Immunization available
Hepatitis C – an infection of the liver caused by HCV. Symptoms are similar to those of HBV but the most common symptom is extreme fatigue	-Contact with blood, body fluids and/or unfixed tissues	-Standard precautions -No vaccine is available
HIV – a serious disease that weakens the body's immune system and destroys the body's ability to fight illness and infection. Symptoms include loss of appetite, weight loss, fever and night sweats, rashes, diarrhea, swollen glands and fatigue. Symptoms may not appear for up to 10 years after initial infection	-Contact with blood, vaginal secretions, semen, breast milk and interior body fluids. There is no evidence that the virus is transmitted through saliva, urine, vomitus, nasal fluid, feces or tears unless blood is visible in these fluids	-Standard precautions -No vaccine is available

Workers may come into contact with blood, body fluids, and/or unfixed tissues by the following routes:

1. ***Needle stick or puncture wound***.
2. ***Broken skin*** in the form of a cut, wound, chapped, abraded, weeping, or rash covered skin that comes into contact with the contaminated item.
3. ***Mucous membranes of the eye, nose, and mouth*** as the result of spatter or aerosolization.

Means to Limit Exposure to BBP's

Engineering Controls:

- The use of specimen containers that are designed to prevent leakage during collection, handling and storage
- Specific biohazard waste containers
- Use of engineered sharps containers
- Use of BSCs when there is any potential for spatter or aerosolization

Administrative Controls:

- Permit system identifying locations and users
- Training (retraining) and authorization of users
- Access control, authorized users only, and other biosecurity measures
- Use of approved labels and signs

Personal Protective Equipment (PPE)

In addition to the standard PPE required for entry to BSL 2 or greater laboratories (Sections 4.3 and 4.4), further PPE is recommended when working with BBP. This includes face protection in the form of face shields or masks in combination with googles or glasses with solid side shields, if there is a potential for splatter or aerosolization. Protective footwear and headwear should also be worn where cross contamination may occur.

Effective Work Practices

It is important that safe work practices are followed when working with BBP, as outlined in Chapter 4.

Good Housekeeping

In addition to the housekeeping recommendations listed below, sterilization and disinfection techniques are discussed further in Chapter 7.

1. Regular cleaning with an appropriate disinfectant.
2. Decontamination with an effective disinfectant when a spill occurs. Allow disinfectant to "sit" on the spill for the recommended contact time.
3. Never pick up contaminated broken glass by hands. Tongs or a brush and dust pan should be used.
4. Contaminated sharps must be placed in an approved and well-marked sharps container.

Vaccination Program

Immunization is available for HBV. Laboratory workers who handle blood, body fluids and/or unfixed human tissue should be vaccinated against HBV. For other work classifications, a detailed risk assessment should be performed to determine whether immunization is recommended.

Standard Precautions

Standard precautions are infection control measures designed to protect workers from diseases spread by blood and body fluids. Underlying these procedures is the fact that the infectious state of the sample or patient is unknown. A prudent approach is to treat all such samples and/or patients as infectious. These standard precautions should be applied when it is difficult to identify the specific body fluid or when body fluids are visibly contaminated with blood.

1. Treat all samples and/or persons as potentially infected when in contact with body fluids.
2. Cover all cuts or abrasions on your skin with a waterproof bandage.
3. Wear gloves.
4. Wash hands with soap and water before and after contact with potentially infectious materials.
5. Clean all potentially contaminated work surfaces with an appropriate disinfectant.
6. Wear protective goggles and a gown if splatter is expected.
7. Do not re-cap needles.
8. Double bag all waste and discard as biological waste.

Chapter 6: Risk Groups and Containment Levels

6.1 Risk Factors

Many of the biological agents used in our research laboratories are pathogenic to humans, animals, or plants. Their use poses risk, which varies with each agent and how it is used. One of the most useful tools available for performing a risk assessment is Public Health Agency of Canada's Pathogen Safety Data Sheets. The simple reference to the risk grouping is not sufficient, and a number of other factors must be taken into consideration, such as those mentioned below and in Table 4:

Pathogenicity/Virulence – Is the pathogen able to infect and cause disease in humans or animals (i.e., pathogenicity)? What is the degree of disease severity in individuals (i.e., virulence)?

Epidemiology – What is the incidence or distribution of a pathogen in a given group of people or geographic area?

Host Range – What are the primary, intermediate, and dead-end hosts? Does the pathogen cause infection in a wide range of species, or is the host range more restricted?

Infectious Dose – What amount of pathogen is required to cause an infection in the host (measured in number of organisms)?

Mode of Transmission – How does the pathogen travel to the host (e.g., direct contact, indirect contact, causal contact, aerosolized droplet or airborne transmission, vectors, zoonosis, intermediate host)?

Incubation Period – What is the period between the infection of an individual by a pathogen and the manifestation of the illness or disease it causes?

Communicability – What is the pathogen's capability of being transmitted from person to person, animal to animal, animal to human, or human to animal?

6.2 Risk Groups

The Public Health Agency of Canada in its *Canadian Biosafety Standards and Guidelines* classifies microorganisms into four different risk groups. These classifications are based upon the World Health Organization (WHO) criteria which in turn are based on the relative hazards of these infective agents. One must keep in mind that the risk group is to be used in the context of laboratory work.

Risk Group 1 Agents
Risk Group 1 agents include microorganisms, nucleic acids, or proteins which are not capable or unlikely to cause disease in healthy workers or animals. RG1 agents also pose a low risk to public health, livestock, or poultry. Many biohazardous materials at Dalhousie fall into this risk group and would include many strains of *Escherichia coli* widely used in molecular biology studies.

Risk Group 2 Agents
Risk group 2 agents are pathogens that pose a moderate risk to the health of individuals or animals, and a low risk to public health, livestock, or poultry. Pathogens that fall into this category are those that can cause human and animal disease, but under normal circumstances are unlikely to do so. Laboratory exposures rarely cause infection leading to serious disease. Included in Risk Group 2 are:

- Bacteria such as *Salmonella enterica, Escherichia coli 0157:H7*
- Viruses such as Hepatitis A,B,C, influenza, measles, mumps, chickenpox

Risk Group 3 Agents
Risk Group 3 agents pose a high risk to the health of individuals and animals, and low risk to public health. Pathogens that fall under this category are those that are likely to cause serious disease in humans and animals. Treatment options and preventive measures are usually available and the risk of spreading the disease caused by these pathogens is low. Depending on the pathogen, the risk of spread to livestock or poultry can range from low to high. Included in this Risk Group 3 are:

- Bacteria such as *Bacillus anthracis* and *Mycobacterium tuberculosis*
- Viruses such as HIV and Yellow fever virus
- Unconventional agents such as Creutzfeldt-Jakob prion

Risk group 4 Agents
Risk group 4 agents pose a high risk to the health of individuals, animals, and public health. Pathogens that fall into this category are likely to cause serious disease which can often lead to death. Effective treatment and preventive measures are not usually available for these pathogens and they may be readily transmitted from one individual/animal to another. Depending on the pathogen, the risk of spread of disease to livestock or poultry can range from low to high. Included in Risk Group 4 are:

- Lassa and Ebola

Table 4. Factors considered when assigning Risk Group to a particular biological agent.

	RG 1	RG 2	RG 3	RG 4
Pathogenicity/Virulence	- Unlikely to cause disease	- Any pathogen that can cause disease but under normal circumstances is unlikely to be a serious hazard to workers, the community, livestock or the environment	- Any pathogen that usually causes serious human disease or can result in serious economic consequences but does not ordinarily spread by casual contact from one individual to another	- Any pathogen that usually produces very serious human disease, often untreatable , and may be readily transmitted from one individual to another, or from animal to human or vice-versa, directly or indirectly, or by casual contact
Infectious Dose	- N/A	- 1000 – 5000 organisms or greater	- 10 – 1000 organisms	- 1 -10 organisms
Mode of Transmission/ Route of Infection	- N/A	- Ingestion, inoculation and mucous membrane route	- Ingestion, inoculation and mucous membrane route. May be transmitted through airborne route, direct contact, vectors	- Readily transmitted, potential for aerosol transmission
Communicability	- N/A	- Limited geographical risk of spread if released from the lab, direct animal-to-animal or human-to-human transmission is relatively limited, limited transmission between different animal species.	- Moderate geographical risk of spread if released from the lab, direct animal-to-animal or human-to-human transmission relatively easy, transmission between animal species may readily occur	- Widespread geographical risk of spread if released from the lab, direct animal-to-animal or human-to-human transmission occurs very easily, transmission between different animal species may occur very readily directly or indirectly or by casual contact
Environmental Stability	- N/A	- Short term survival, can survive under ideal conditions	- Resistant (days to months)	- Highly resistant (months to years)

	RG 1	RG 2	RG 3	RG 4
Host Range	- N/A	- Infects a limited number of species	- Infects multiple species	- Infects many species
Endemicity	- Enzootic	- Generally enzootic	- Exotic or enzootic but subject to official control	- Exotic
Economic/Public Health Aspects	- No economic and/or clinical significance	- Limited economic and/or clinical significance	- Severe economic and/or clinical significance	- Extremely severe economic and/or clinical significance
Prophylaxis	- N/A	- Effective treatment and preventive measures are available	- Prophylactic and/or therapeutic treatments may or may not be readily available	- Prophylactic and/or therapeutic treatments are not usually available
Vectors	- N/A	- Does not depend on vectors or intermediate hosts for transmission	- May depend on vectors or intermediate hosts for transmission	- May depend on vectors or intermediate hosts for transmission
Recombinants	- Recombinant is RG 1, the modifications have not changed the risk	- Recombinant is RG 2, the modifications have not changed the risk; DNA from a RG 2 or RG 3 organism is transferred into a RG 1 organism but not the whole genome; recombinant is a RG 3 or RG 4 organism but the modification has resulted in proven attenuation	- Recombinant is a RG 3 organism; the modifications have not changed the risk; recombinant is based on a RG 2 organism however the modifications have increased the risk	- Recombinant is a RG 4 organism; the modifications have not changed the risk; DNA from RG 4 is transferred into RG 1 organism in absence of demonstration of lack of virulence or pathogenicity.

6.3 Relationship of Risk Groups with Biosafety Levels

The relationship of risk groups with biosafety levels, practices, and equipment is shown in Table 5. Experience has demonstrated the prudence of the Biosafety Levels 1-4 practices, procedures, and facilities for manipulations of biohazards in laboratory and animal care settings.

Risk implies the probability that harm, injury, or disease will occur. In the context of laboratories using biohazardous materials the assessment focuses primarily on the prevention of laboratory acquired infections. This table was developed with reference to information from the PHAC's *Canadian Biosafety Standards and Guidelines,* WHO's *Laboratory Biosafety Manual,* CDC's *Biosafety in Microbiological and Biomedical Laboratories*, and NIH's *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.*

Table 5. Biosafety level, work practices, and safety equipment required for Risk Group 1-4 biological agents.

Risk Group	Biosafety Level	Laboratory Type	Laboratory Practices	Safety Equipment
1	- Basic-Biosafety Level 1	- Basic teaching, research	-GMT*	- None, open bench work
2	- Basic-Biosafety Level 2	- Primary health services; diagnostic, research	-GMT* plus protective clothing, biohazard sign	- Open bench plus BSC for potential aerosols
3	- Containment-Biosafety Level 3	- Special diagnostic, research	-As Level 2 plus special clothing, controlled access, directional air flow	- BSC and/or other primary devices for all activities
4	- Maximum containment-Biosafety Level 4	- Dangerous pathogen units	-As Level 3 plus airlock entry, shower exit, special waste disposal	- Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave, filtered air

* GMT – Good microbiological technique

6.4 Laboratory Acquired Infections

Laboratory acquired infections (LAIs) are described as direct or indirect infections following exposure to an infectious biological agent in a laboratory, which may be symptomatic or asymptomatic. Infectious agents may enter the body by ingestion, inhalation, puncture, or absorption. Underlying health issues may alter host defense and increase the risk of acquiring a laboratory infection. LAIs can be transmitted to others within or outside the laboratory setting.

It is not easy to define a laboratory acquired infection or to conclude with certainty that one has occurred, particularly when the pathogen in question is also found in the community. Even with biosafety programs in place LAIs are not uncommon, with 107 cases and 9 deaths reported from 2000 to 2004 (Table 6) [2]. There is a lack of precise data on the occurrence of LAIs, as many incidents are unreported or go undetected.

Table 6. Reported cases of symptomatic LAIs from 2000 through 2004

Category of Agent	Infections	Deaths
Bacteria*	77	7
Rickettsiae	2	0
Viruses	26	2**
Parasites	1	0
Fungi	1	0
Total	**107**	**9**

* Includes chlamydial infections
** 1 death associated with secondary-exposure

Risk assessment, PPE, and safe work practices can minimize the risk of contracting a LAI. All suspected or potential cases of LAIs should be reported. In addition, any incidents that have the potential to cause an LAI must be reported and investigated.

[2] Fleming, Diane O. Hunt, Debra L. (2006). Biological Safety - Principles and Practices (4th Edition). (pp. 53-70). American Society for Microbiology (ASM).

Chapter 7: Biosafety Cabinets

A biosafety cabinet is a ventilated cabinet which uses a variety of combinations of HEPA filtration, laminar air flow, and containment. The purpose is to provide protection from particulates or aerosols involving biohazardous materials to personnel, products, and the environment. These cabinets are to be used when infectious materials are being exposed in open containers, when there is an increased risk of airborne infection, and when there is a high probability of generating contaminated aerosols. It is distinguished from a chemical fume hood by the presence of HEPA filtration and the laminar nature of the air flow.

HEPA filters trap 99.97% of particles of 0.3 μm in diameter and 99.99% of particles of greater size. This enables the HEPA filter to effectively trap all known infectious microbes and ensure that only safe air is discharged from the cabinet. HEPA filtered air is directed over the work surface providing protection of work surface materials from contamination. There are three classes of BSCs. The type of protection provided by the different classes of BSCs is outlined in Table 7. Differences in face velocity, air flow, and exhaust systems are outlined in Table 8.

Table 7. Selection of BSC based on type of protection needed.

Type of Protection	BSC Selection
Personnel protection from Risk Groups 1-3 microorganisms	Class I, Class II, Class III
Personnel protection from Risk Group 4 microorganisms, glove box laboratory	Class III
Personnel protection from Risk Group 4 organisms, suit laboratory	Class I, Class II
Product protection	Class II, Class III if laminar flow included
Volatile radionuclide/chemical protection, minute amounts	Class IIA2, Class IIB1, Class IIB2 vented to the outside
Volatile radionuclide/chemical protection	Class I if hard ducted, Class IIB2, Class III

Table 8. Differences in the face velocity, recirculated air flow, and exhaust systems between Class I, II and III BSCs.

BSC	Air Flow Face Velocity (m/s)	% of air flow		Exhaust System
		Recirculated	Exhausted	
Class I	0.38	0	100	Hard duct or exhaust to room
Class IIA1	>0.38	70	30	Exhaust to room or thimble connection to outside atmosphere
Class IIA2 vented outside	>0.51	70	30	Exhaust to room or thimble connection to outside atmosphere
Class IIB1	>0.51	<50	>50	Hard duct
Class IIB2	>0.51	0	100	Hard duct
Class III	A maintained face velocity of 0.7m/s when one glove is removed	0	100	Hard duct

7.1 Class I

Class I BSCs (Figure 8) provide protection to the worker and environment but no protection to the work surface or products. Class I BSCs are used to enclose equipment or for procedures where product protection is not a concern.

Air passes over the work surface and is discharged from the cabinet through the exhaust duct and a HEPA filter. The directional flow of air whisks aerosol particles generated on the work surface away from the worker and into the exhaust duct. The front opening allows the operator's arms to reach the work surface inside the cabinet while the worker observes the work surface through the glass window. The window can be raised for cleaning purposes.

The air from the cabinet is exhausted through a HEPA filter in one of three ways:

1. Into the laboratory and then to the outside through the building exhaust.
2. Directly to the outside through the building exhaust.
3. Directly to the outside through a dedicated exhaust duct.

The HEPA filter may be located in the exhaust plenum of the BSC or the building exhaust.

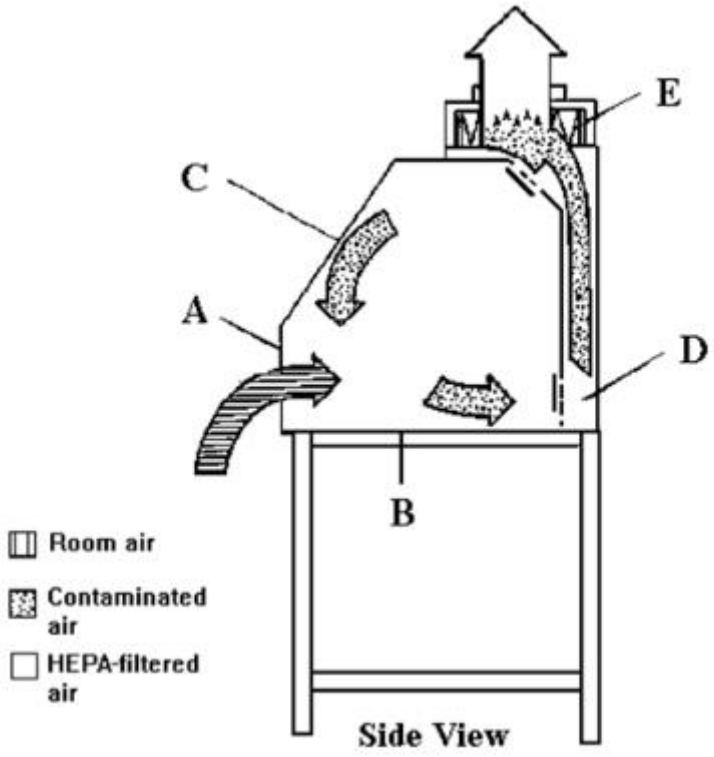


Figure 8. Class I BSC (A: front opening, B: work surface, C: window, D: exhaust plenum, E: exhaust HEPA filter).

7.2 Class II

Class II BSCs (Figures 9 and 10) are designed to provide personnel and environment protection, but they also protect work surface materials from contaminated room air. Class II BSCs differ from Class I in that they allow only HEPA filtered air to flow over the work surface. Supply air is drawn downwards away from the work surface, passing though the HEPA filter prior to passing over the work surface. There are four subtypes of Class II cabinets: A1, A2, B1, and B2. These differ from one another by:

1. Air intake velocity.
2. Amount of air recirculated over the work surface.
3. Type B2 cabinets are hard ducted to a dedicated external exhaust.

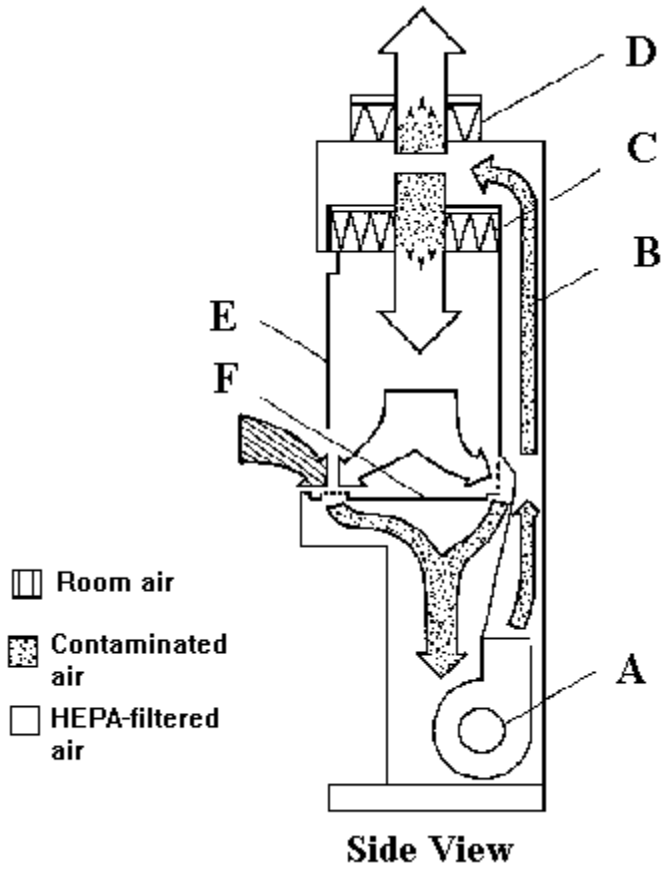


Figure 9. Class II Type A BSC (A: blower, B: rear plenum, C: supply HEPA filter, D: exhaust HEPA filter, E: sash, F: work surface).

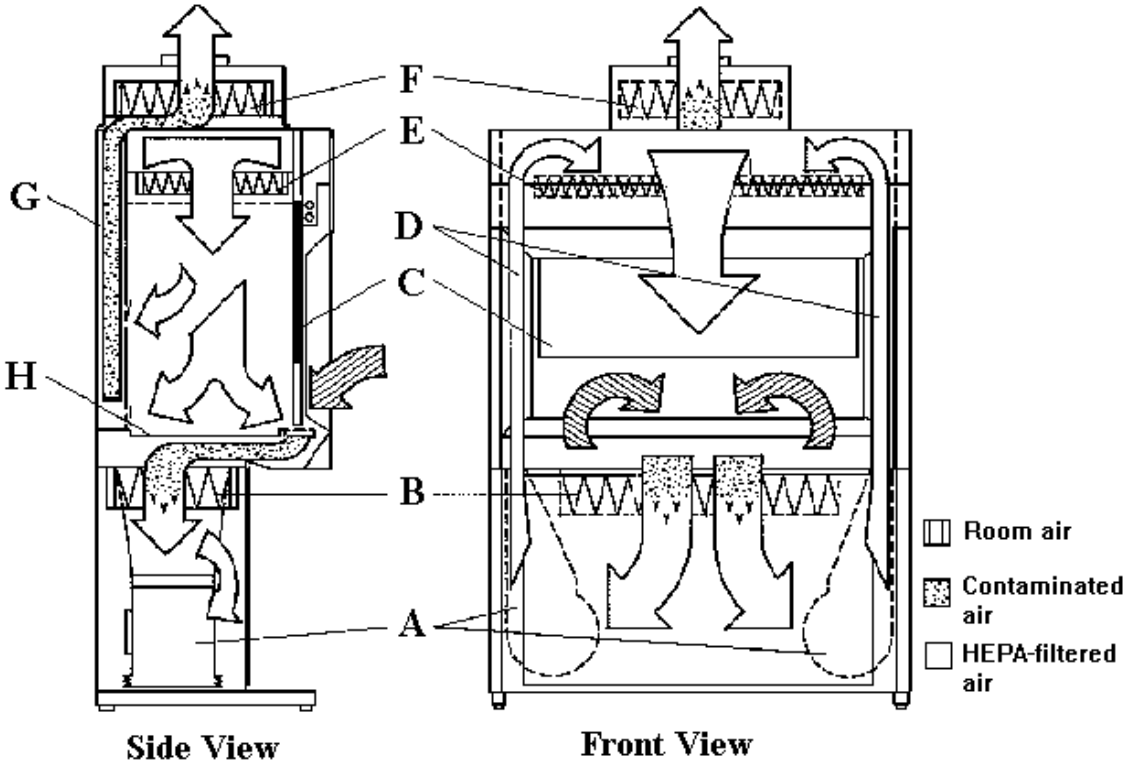


Figure 10. Class II Type B1 BSC (A: blower, B: supply HEPA filter, C: sash, D: HEPA filtered air, E: supply HEPA filter, F: exhaust HEPA filter, G: negative pressure exhaust plenum, H: work surface).

7.3 Class III BSC

Class III cabinets (Figure 11) offer product protection and the highest level of personal protection. Class III BSCs must be used for Risk Group 4 agents and can be an alternative to positive-pressure suits if the material is exclusively handled within the BSC. All penetrations are sealed “gas tight”. Both air supply and exhaust are HEPA filtered. Air flow is maintained by a dedicated exhaust system exterior to the cabinet, which keeps the cabinet interior under negative pressure. Access to the work surface is by means of heavy duty rubber gloves, attached to ports in the cabinet. The cabinet has an attached sterililizable pass-through box. The Class III cabinet may be connected to a double door autoclave, dunk tank, or a bag-in/bag-out system used to decontaminate all materials entering or leaving the cabinet.

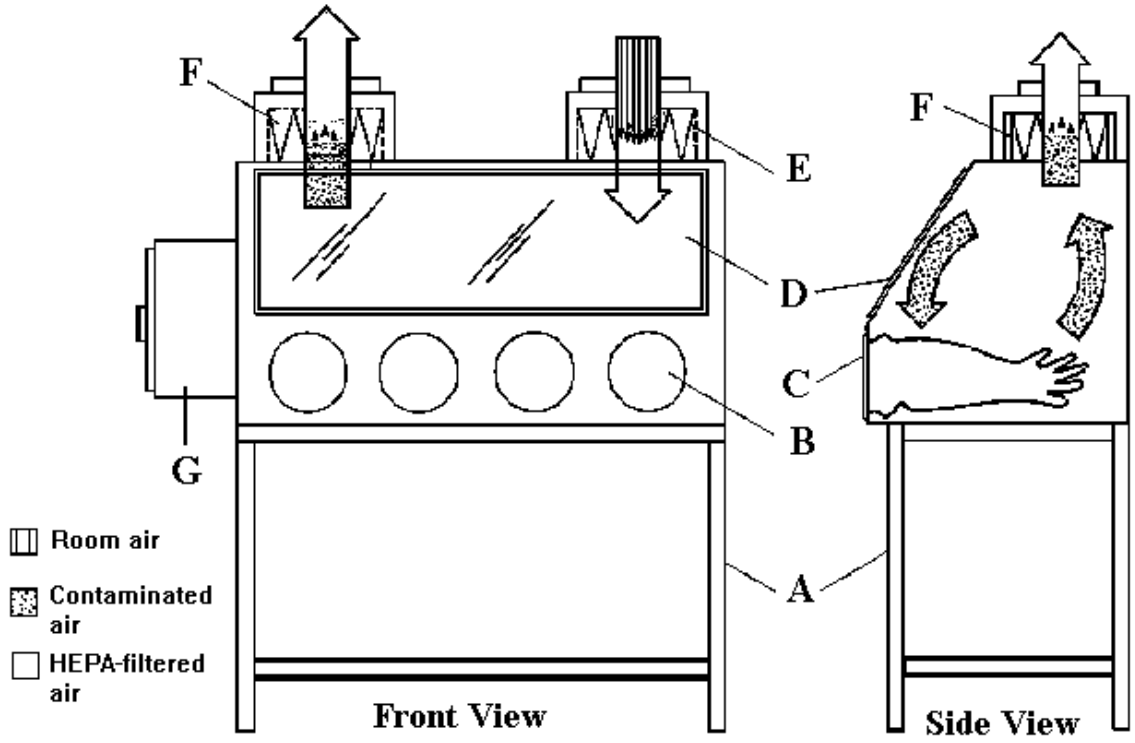


Figure 11. Class III BSC (A: BSC supports, B & C: glove ports, D: sash, E: supply HEPA filter, F: exhaust HEPA filter, G: double door autoclave/pass-through box).

7.4 Effective Use of BSCs

1. Read the operator's manual and follow manufacturer's recommendations.
2. Locate the cabinet in an area where it will not be adversely affected by air currents, and is away from pedestrian traffic and other ventilation devices.
3. The use of UV light to decontaminate the BSC is not encouraged. If the UV light is in use, turn it off before commencing work in the BSC.
4. Turn on the fluorescent light and cabinet blower. Allow the BSC to run 15 minutes before using.
5. Wash hands thoroughly before proceeding and wear required personal protective equipment. If laboratory coats rather than gowns are worn, they must be buttoned and the gloves pulled over the wrist of the coat.
6. Workers should double glove when working in the BSC. The outer layer of gloves must be removed after completion of work ***PRIOR*** to removing their hands from the BSC.
7. Wipe down the interior surfaces with 70% ethanol (or other suitable disinfectant) and allow to dry.
8. Arm movement into and out of the cabinet should be kept to a minimum to avoid air turbulence. Place all materials needed for the procedure inside the cabinet prior to starting. Do not bring any unnecessary items into the cabinet. If it is necessary to move arms in and out of the cabinet, do so slowly. Arms should enter/exit the BSC perpendicular to the front opening.
9. Minimize air changes in the room by avoiding opening and closing laboratory doors and pedestrian traffic.
10. Work at least 10 – 15 cm from the opening of the cabinet. Objects should not be placed such that they obstruct the front or rear grilles.
11. Adjust stool so that the worker's face is above the front opening of the cabinet. The stool height should be such that the sash is level with the underarms of the worker.
12. Delay manipulation of materials for at least one minute after putting arms in the cabinet to allow the cabinet environment to stabilize.
13. Carry out work on a plastic-backed absorbent pad to contain small spills, making sure that this pad does not cover the front grille opening.
14. ***The use of open flames is prohibited in the BSC*** as they disrupt the air flow patterns and may damage the HEPA filter. To sterilize transfer loops, electronic loop incinerators, microincinerators, or disposable loops are an alternative.
15. The use of germicidal lamps is strongly discouraged. Their germicidal properties are not highly effective and there is the concern for inadvertent UV exposure to the eyes.
16. Clean up spills as soon as they occur.
17. Place contaminated items to the rear of the cabinet.
18. Materials should be discarded in a waste container located towards the rear of the BSC. Do not discard items in a container that is located outside of the BSC.
19. Disinfect the cabinet after use. A bottle of appropriate disinfectant should be kept in the BSC to avoid having to move hands out of the BSC.
20. ***NEVER*** attempt to remove or change the HEPA filters.

21. Leave the fan blower on in the cabinet for five minute after you have finished your procedure to allow the system to purge.

BSC Certification

New BSCs must be certified by an approved certification company upon installation and before use. BSCs are not to be used without certification. BSCs must be recertified annually, or if repairs are conducted, or if they are moved to a new location

Chapter 8: Sterilization and Disinfection

It is important to ensure that biohazardous materials involved in research are inactivated during spill clean-up, before cleaning equipment for re-use, and before final disposal. Microorganisms vary in their resistance to destruction by physical and chemical means. It is important to distinguish between *sterilization, disinfection,* and *decontamination*.

Sterilization
The process of treating an object or material to remove or kill ***all*** living organisms.

Disinfection
The process of killing pathogenic agents by chemical or physical means. Disinfection does not necessarily involve the total destruction or removal of all organisms. Sterile conditions may not necessarily be present.

Decontamination
The process of reducing the number of microorganisms to an acceptable level. Decontamination can be achieved by either disinfection or sterilization.

Whether or not sterility is achieved is dependent on the following factors:
- Type and number of microorganisms
- Concentration of the agent
- Length of contact time with the agent
- Presence of organic matter and dirt
- Temperature
- Condition and nature of the surface

Sterilizing and disinfecting agents work in various ways. They may injure the cell membrane, altering the normal selective permeability. Damaged cell membranes may allow metabolically important components to escape or prevent entry of nutrients. Sterilizing or disinfecting agents may react with a specific cellular enzyme to prevent it from reacting with its natural substrate. Some disinfectants, as well as the moist heat in autoclaves, coagulate or denature protein and render the cell non-functional.

Most bacteria, fungi, and lipid-containing viruses are relatively susceptible to chemical decontamination. Non-lipid containing viruses and bacteria with a waxy coat occupy mid-range of resistance. Spores of spore-formers are the most resistant to decontamination.

The following methods are used to achieve sterilization and disinfection.
- Steam sterilization (autoclaves)
- Dry heat
- Gas sterilization
- Ultraviolet lamps
- Chemical disinfectants

8.1 Autoclaves

The autoclave is a common piece of equipment found in many laboratories or laboratory associated facilities. The purpose of the autoclave is to render treated material sterile (i.e. free of any living organisms) prior to disposal. There are three basic autoclave cycles:

Gravity or *"fast exhaust"* cycle is used to sterilize dry goods, glassware, etc. This cycle charges the chamber with steam and holds it at a set temperature for a set period of time. At the end of the cycle a valve opens and the chamber rapidly returns to atmospheric pressure. Drying time may also be added to the cycle.

Liquid or *"slow exhaust"* is used to prevent sterilized liquids from boiling. Steam is exhausted slowly at the end of the cycle, allowing the super-heated liquids to cool.

Pre-vacuum cycle is used for porous materials. This cycle partially evacuates the chamber prior to introducing steam for greater steam penetration. Pre-vacuum cycles are not available on all machines.

Like a pressure cooker, the autoclave operates by using steam under pressure (Figure 12). High pressure steam can reach high temperatures, thus increasing its heat content and sterilizing power. Most of the heating power of steam comes from its latent heat of vaporization, i.e. the amount of heat required to convert boiling water to steam. Steam is able to penetrate objects with cooler temperatures because once the steam contacts a cooler surface, it immediately condenses to water. This creates negative pressure at the point of condensation and draws more steam to the area. Condensation continues as long as the temperature of the condensing surface is less than that of the steam. These properties ensure rapid heating of surfaces, good penetration of dense materials, and coagulation of proteins.

The death rate of microorganisms is directly proportional to the concentration of microorganisms at a given time. The time required to kill a known population of microorganisms in a specific suspension at a particular temperature is referred to as thermal death time (TDT). Increasing the temperature decreases TDT, and lowering the temperature increases TDT. Environmental conditions also influence TDT. Increased heat causes increased toxicity of metabolic products and toxins. TDT decreases under conditions of pronounced acidic or basic pH. Fats and oils slow heat penetration and increase TDT.

Longer autoclaving times are required for large loads, large volumes of liquid, and more dense materials. Autoclaving is an ideal technique for sterilizing biohazard waste, surgical dressings, glassware, microbiological media, and liquids. It is not good for treating animal carcasses, furniture, and equipment sensitive to heat and moisture. Autoclaving should not be used to sterilize radioactive materials or volatile toxic chemicals.

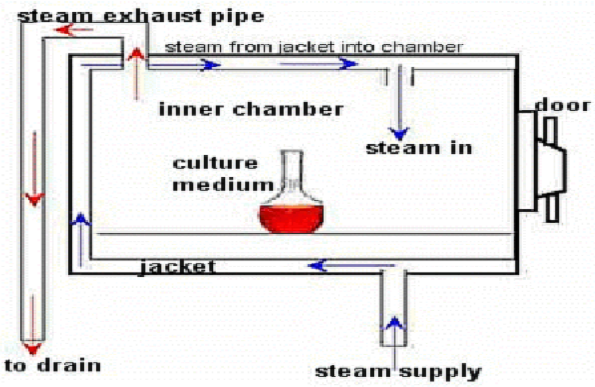


Figure 12. Schematic representation of steam flow in an autoclave.

Elements Required for Effective Autoclave Use

Temperature

Minimum chamber temperature of 121°C.

Time

Minimum autoclaving time is measured ***after*** the temperature reaches 121°C and pressure reaches 15 psi. See Table 9.

Contact

Steam saturation of the load is essential. Air pockets or insufficient steam prevent adequate contact.

Containers

Use leak-proof containers for items to be autoclaved. Place plastic bags inside a secondary container in the autoclave to catch any leaks. Plastic and stainless steel make appropriate secondary containers. Ensure that plastic bags and pans are ***autoclavable***, to avoid having to clean up melted plastic.

Indicators

Tape indicators can only verify that the autoclave has reached normal operating temperatures. Most chemical indicators change colour after being exposed to a temperature of 121°C, but cannot measure the length of time at this temperature. Biological indicators such as *Bacillus stearothermophilus* spore strips and certain chemical indicators verify that the autoclave reached adequate temperature for a long enough time to kill the microorganisms.

Efficacy testers

Spore testing is conducted monthly.

Autoclave Safety Practices

1. Do not exceed the manufacturer's recommended pressures and temperatures.
2. Arrange for regularly scheduled inspections/testing of autoclaves and ancillary equipment.
3. Report all malfunctions and tag the unit "Out of Service".
4. Ensure that the operational SOP is posted near the autoclave.
5. Do not use the autoclave unless you have specific training in safe operating procedures.
6. Ensure that your name is listed on the authorized user list, which must be posted in the vicinity of the unit.
7. Clean the drain strainer before loading the autoclave.
8. Load the autoclave as per the manufacturer's instructions.
9. Make the appropriate log entry.
10. Loosen caps on containers of liquids before loading to avoid having the bottles shatter during pressurization.
11. Use a tray with a solid bottom and walls as double containment to catch spills.
12. Add approximately 1 cm of water to the trays so that the bottles will heat evenly.
13. Do not load plastics that are incompatible with the autoclave.
14. Do not stack containers.
15. Do not overload the autoclave.
16. Firmly lock the autoclave doors prior to starting the run to prevent the sudden release of high pressure steam.
17. Ensure that the correct cycle is selected.
18. Before opening the autoclave door after the run and unloading the autoclave, wear a rubber apron, rubber sleeve protectors, heat resistant gloves, and a face shield.
19. Release the steam slowly, as bottle plugs may be ejected if the pressure is released too quickly.
20. Stand so the autoclave door shields your body from the contents of the unit and released steam while opening the autoclave.
21. Wait 5 minutes for loads containing dry glassware and 10 minutes for liquid loads before removing items. Vessels containing liquid volumes in excess of 20 litres should be allowed to cool in the autoclave before being unloaded as superheated liquids continue to boil for some time.
22. Allow glassware to cool completely before handling with ungloved hands.
23. Remove debris from the autoclave that could block drain valves and create a hazard for the next user.
24. Instructions for autoclaving biohazardous waste are outlined in Dalhousie University's Policy for Management of Biological Waste (Appendix 5).

Principal investigators/supervisors must be aware that autoclaves are not to be used without certification. Autoclaves are to be recertified when a failure is indicated in efficacy testers.

As there are a number of brands and types of autoclaves, it is the responsibility of the principal investigator/laboratory supervisor for users to be trained in the specific type of autoclave in use. As a general rule, the following table can be used as a guide for preparation of items for autoclaving. Specific techniques, where temperature and pressure vary, may be defined by the investigator.

Table 9. Guide for autoclaving biohazardous waste

Program	Temperature	Minimum Time	Drying Time	Items to be Sterilized
Liquids	121°C	15 min	None	Distilled water, solutions, media
Unwrapped	135°C	3 min	Default 15 min	Unwrapped instruments, metal, glass, plastic, heat-resistant rubber tubing
Wrapped	135°C	10 min	Default 15 min	Bandages, pads, wrapped instruments
Packs	121°C	30 min	Default 15 min	Groups of instruments in commercially prepared packs, instruments subject to prolonged storage
Optional	User defined from 100°C – 136°C	User defined	User defined 1-99 min	Items appropriate to user defined parameters

8.2 Ultraviolet Lamps

Ultraviolet (UV) light is electromagnetic radiation in the spectral region from less than 200 nanometres to 400 nanometres (nm) (Figure 13). Ultraviolet light is that portion of the electromagnetic spectrum that lies between the “purple” edge of the visible spectrum and x-rays. Intense or prolonged exposure to UV light can result in painful eye injury (photokeratitis, retinal burns), skin burns, premature aging of the skin, and skin cancer. Shielding and PPE should be employed. Regular glass does not afford complete protection from the harmful effects of UV light.

UV radiation is divided into several regions: UV-A (315-400 nm); UV-B (280-315 nm); UV-C (200-280 nm) and vacuum UV (40-200 nm). Germicidal lamps used in laboratory settings emit UV-C radiation. It is recommended that the time of exposure to an intensity of 100 micro watts per square centimetre at a wavelength of 254 nm not exceed one minute.

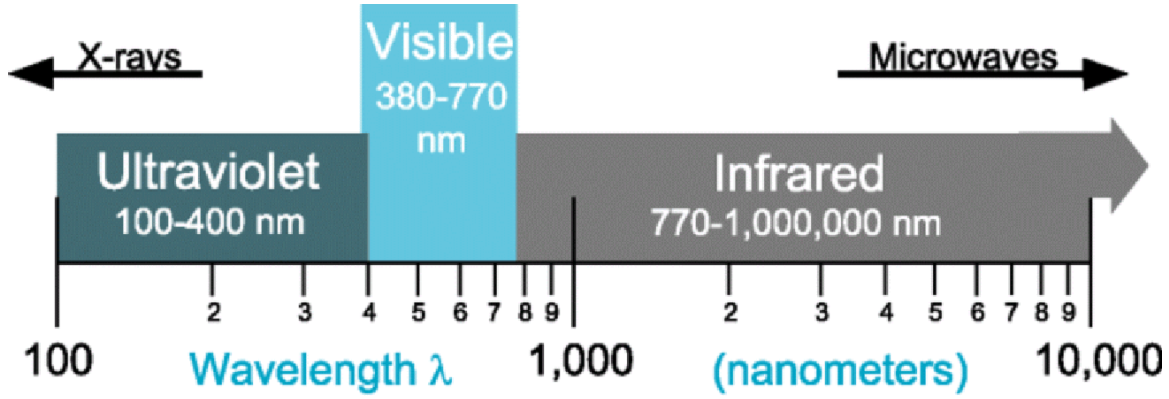


Figure 13. UV Light is a portion (100-400nm) of the electromagnetic spectrum.

Common UV generating devices are outlined in the table below, including their common uses and PPE requirements.

Table 10. Ultraviolet light generating devices in a university setting with their personal protective equipment and maintenance requirements.

Device	Uses	PPE Requirements	Maintenance and Monitoring
Transilluminator	Visualizing nucleic acids following gel electrophoresis and ethidium bromide staining	Gloves, lab coat, UV light face shield	As per manufacturer's instructions
Hand-held UV units	Visualizing nucleic acids following gel electrophoresis and ethidium bromide staining	Gloves, lab coat, UV light face shield	As per manufacturer's instructions
Germicidal lamps in Biosafety Cabinets*	In conjunction with chemical disinfection, disinfection of the interior surfaces of the biosafety cabinet prior to and after use	Gloves, lab coat, UV light face shield	Clean UV light bulb on a monthly basis and replace when necessary as per manufacturer's instructions
Germicidal lamps in laboratories	For air and surface disinfection. University *Hazard Identification* warning labels must be posted	No entry when lamp is operating	Wipe UV light bulbs on a monthly basis

*The use of germicidal lamps to disinfect BSCs is discouraged

UV Light Protective Measures

1. Wear PPE, which would include gloves, laboratory coat, goggles, and face shields.
2. Post a UV light symbol where UV light sources are operating at a wavelength capable of germicidal irradiation are present.
3. Limit exposure times.

Symptoms of UV Light Overexposure

- Skin: Sunburn-like symptoms.
- Eyes: Burning painful sensation, sensitivity to light, sensation of a foreign object (sand) in the eye, and tearing.

These symptoms usually develop several hours after overexposure to UV light has occurred. Medical attention should be sought immediately, especially if the eyes are involved.

8.3 Chemical Disinfectants

Unlike steam sterilization, it is feasible to use chemical disinfectants (Table 11) in large spaces, on surfaces and stationary equipment, and on delicate instruments which might be damaged by high temperatures and moisture.

Chemical disinfectants are a double-edged sword. Although their use is necessary in many routine laboratory and health care settings, the ability of these products to kill infectious agents also makes them potentially harmful to humans and the environment.

There are many trade names for the wide variety of disinfectants. Typical active ingredients fall into the following categories:

- Acids/alkalis
- Alcohols
- Oxidizers (e.g., bleach, iodine)
- Aldehydes
- Metals
- Phenols
- Quaternaries

The relative resistance to chemical disinfectants can be substantially altered by the following factors:

- Contact time
- Human error
- Concentration
- Presence of organic matter and dirt
- Temperature
- Humidity
- Types and numbers of microorganisms
- Condition and nature of the surfaces

Refer to the Material Safety Data Sheet (MSDS) and Pathogen Safety Data Sheet (PSDS) to see what chemical disinfectants are effective against the agent you are working with.

When using diluted bleach solution as the chemical disinfectant, be sure to check the concentration of the bleach in the supply bottle. Bleach is not supplied at the same concentration from all manufacturers, and you may unknowingly prepare a more dilute bleach solution than what is effective against the pathogen with which you are working.

As many of these chemical disinfectants are at the least a skin irritant and others are fairly toxic, care must be taken to avoid skin exposure and/or ingestion. Appropriate PPE when using these agents would include laboratory coats, gloves, and eye or face shields if there is any likelihood of splashes.

Table 11. Summary of the properties of a selection of chemical disinfectants.

Chemical Agent	Antiseptic or Disinfectant	Mechanism of Activity	Applications	Limitations	Antimicrobial Spectrum
Chlorine	- Chlorine gas - Sodium hypochlorite - Chloramines	- Protein oxidation - Membrane leakage	- Water treatment - Skin antisepsis - Equipment spraying	- Inactivated by organic matter - Objectionable odour	- Broad variety of bacteria, fungi, protozoa and viruses
Iodine	- Tincture of iodine - Iodophors	- Halogenates tyrosine in protein	- Skin antisepsis - Preoperative preparation	- Inactivated by organic matter - Objectionable odour	- Broad variety of bacteria, fungi, protozoa and viruses
Phenol and derivative	- Cresols - Trichlosan - Hexachlorophene - Hexylresorcinol - Chlorhexidine	- Coagulates protein - Disrupts cell membranes	- General preservatives - Skin antisepsis with detergent	- Toxic to tissue - Disagreeable odour	- Gram-positive bacteria - Some fungi
Mercury	- Mercuric chloride - Merthiolate - Metaphen	- Combines with -SH groups in proteins	- Skin antisepsis - Disinfectants	- Inactivated by organic matter - Toxic to tissue - Slow acting	- Broad variety of bacteria, fungi, protozoa and viruses
Copper	- Copper sulphate	- Combines with proteins	- Algicide in swimming pools - Municipal water supplies	- Inactivated by organic matter	- Algae - Some fungi
Silver	- Silver nitrate	- Binds proteins	- Skin antiseptic	- Skin irritation	- Organisms in burned tissue - Gonococci
Alcohol	- 70% ethyl alcohol	- Denatures proteins - Dissolves lipids - Dehydrating agent	- Instrument disinfectant - Skin antiseptic	- Precleaning necessary - Skin irritation	- Vegetative bacterial cells, fungi, protozoa and viruses

Chemical Agent	Antiseptic or Disinfectant	Mechanism of Activity	Applications	Limitations	Antimicrobial Spectrum
Formaldehyde	- Formaldehyde gas - Formalin	- Reacts with functional groups in proteins and nucleic acids	- Embalming - Vaccine production - Gaseous sterilization	- Poor penetration - Allergenic - Toxic to tissues - Neutralized by organic matter	- Broad variety of bacteria, fungi, protozoa and viruses
Ethylene oxide	- Ethylene oxide gas	- Reacts with functional groups in proteins and nucleic acids	- Sterilization of instruments, equipment, heat-sensitive objects	- Explosive - Toxic to skin - Requires constant humidity	- All microorganisms, including spores
Glutaraldehyde	- Glutaraldehyde	- Reacts with functional groups in proteins and nucleic acids	- Sterilization of surgical supplies	- Unstable - Toxic to skin	- All microorganisms, including spores
Hydrogen peroxide	- Hydrogen peroxide	- Creates aerobic environment - Oxidizes protein groups	- Wound treatment - Room decontamination in vapour form	- Limited use - requires specialized equipment to use vapour form	- Anaerobic bacteria
Cationic detergents	- Commercial detergents	- Dissolve lipids in cell membranes	- Industrial sanitization - Skin antiseptic - Disinfectant	- Neutralized by soap	- Broad variety of microorganisms
Triphenyl-methane dyes	- Malachite green - Crystal violet	- React with cytoplasmic components	- Wounds - Skin infection	- Residual stain	- Staphylococci - Some fungi - Gram-positive bacteria
Acridine dyes	- Acriflavine - Proflavine	- React with cytoplasmic components	- Skin infection	- Residual stain	- Staphylococci - Gram-positive bacteria
Acids	- Benzoic acid - Salicylic acid - Undecylinic acid - Lactic and propionic acids	- Alter pH	- Skin infections - Food preservative	- Skin irritation	- Many bacteria and fungi

8.4 Laboratory Spills

Biohazard Spill Kit

Each laboratory where biohazardous materials are present should have the appropriate equipment and supplies on hand to manage spills and accidents involving biohazardous materials. Permanent equipment should include an eyewash station, a hand-washing sink, and spill kit supplies. Items to be included in your spill kit should include:

- Clean up instructions
- Disposable gloves
- Laboratory coat (preferably disposable)
- Safety goggles
- Dust mask
- Disposable shoe covers
- Absorbent materials - paper towels, granular absorbent material
- All purpose disinfectant
- Autoclave bucket for diluting disinfectant
- Tongs, forceps
- Dust pan and small broom
- Sharps container
- Autoclave biohazard waste bags
- Biohazardous spill warning signs

Spill Clean-Up Procedure (BSL 1, BSL 2)

Laboratories using biohazardous materials should develop a spill response plan addressing foreseeable occurrences. ***Personal exposure takes priority over clean up.*** If personnel are exposed, immediately remove contaminated clothing and other protective equipment, and wash affected areas with soap and water. If medical follow up is warranted it should be sought immediately. Submit an incident report as per section 8.5.

Procedure

1. Alert personnel and clear the immediate area. Cordon off the area to prevent others from entering. Allow 30 minutes for the spill aerosols to settle before proceeding with spill clean-up.
2. If the spill has splattered the face, flush eyes and face with tepid water for a minimum of 15 minutes. Remove any contaminated clothing.
3. Put on personal protective equipment.
4. Cover an area twice the size of the spill with disinfectant-soaked paper towel, or surround the spill with dry disinfectant as per label instructions. Apply disinfectant working from the outside of the spill towards the centre.
5. Allow 30 minutes of contact time.
6. Wipe down any contaminated equipment or furniture with disinfectant.
7. Place any materials that came into contact with spill in a biohazard bag.
8. Use forceps, tongs, or broom to remove broken glass and other items.

9. Place sharps in an appropriate container
10. Remove towels and re-clean area with a disinfectant solution. Spills should be cleaned from the outside towards the centre.
11. Decontaminate re-usable clean up items and other re-usable equipment.
12. Remove personal protective equipment and place in an autoclave bag for sterilization. Wash hands thoroughly (Section 4.4, Figure 3).
13. Inform personnel when clean-up is complete.
14. Replenish the spill kit.
15. Prepare an incident report for submission to the Biosafety Officer.

Clean-up Procedures for BSCs

1. ***Keep the cabinet running***
2. All materials inside the BSC must be cleaned with disinfectant prior to being removed from the BSC.
3. Clean up as per above procedure, ensuring that you wipe down the back and side walls of the cabinet.
4. Fill the basin with disinfectant to capacity if material has spilled in the catch basin, wait 30 minutes, and then absorb with paper towels.
5. Allow cabinet to run for 10 minutes after clean-up is complete before resuming work in the BSC.

Clean-up Procedures for Centrifuges

1. Shut centrifuge off and do not open lid for 20 minutes to allow aerosols to settle.
2. Put on personal protective equipment.
3. Use a squeeze bottle to apply disinfectant to all contaminated surfaces, taking care to minimize splashing.
4. Allow 20 minute contact time and then complete clean-up of the chamber.
5. Remove buckets and rotors to nearest BSC for disinfection.

Spills Outside of the Laboratory

1. Viable organisms transported outside the laboratory must be in a well-sealed primary container enclosed in a secondary container with a closable lid.
2. Wipe down the exterior of the secondary container with disinfectant before leaving the laboratory so that it can be transported without gloves.
3. Carry paper towels should a spill occur.
4. Notify people in the immediate area of a spill and gather the necessary materials to proceed with the clean-up.

8.5 Incident Reporting

An incident is an unplanned, unwanted event that results in or could have resulted in harm to people, property, or the environment. An incident without harm is referred to as a near miss. Incidents, including near misses, are required to be reported to the Environmental Health & Safety Office.

It is important to understand that the purpose of reporting incidents is not to find fault with the worker. Reporting incidents allows the root cause to be identified and rectified so that a repeat incident does not occur. Root causes of incidents may include inadequate safe work procedures or inadequate training of personnel. Identifying the root cause will allow for improvement of the Biosafety Program. Reporting near misses is important as it identifies situations that may cause harm in the future and which could therefore be prevented.

Incidents should be reported immediately to the laboratory supervisor. The supervisor will complete Dalhousie University's Accident/Incident Report, which is available either online or in hard copy from the Environmental Health & Safety Office. Once completed, the original report is to be submitted to the Environmental Health & Safety Office. Copies of the report are to be given to the person involved in the incident, the supervisor, and the local safety committee. If the incident involved an explosion or resulted in a fatality, the accident/incident report must be submitted within 24 hours of the occurrence of the incident. Other incidents should be reported to the Environmental Health & Safety Office with 48 hours of occurrence.

Chapter 9: Transportation of Biological Agents

Handling, transport, and shipment of biological materials is governed by law, and proper care is absolutely essential. Transportation methods must minimize risks to employees of the carrier, the public, and the staff of the receiving laboratory. Hazards are compounded by improper packaging. In Canada, courier shipments are required to meet Transportation of Dangerous Goods (TDG) regulations as well as International Air Transport (IATA) regulations. Individuals who plan to transport or ship biological agents are required to complete the appropriate training.

Under the Transportation of Dangerous Goods regulations, biohazardous materials fall under ***Class 6.2 Infectious*** (substances which are known or suspected to cause disease, may be hazardous to animals or humans or both). The appropriate shipping label for biohazardous materials is shown in Figure 14.

Figure 14. Biohazard shipping label.

There are six main categories under IATA and TDG Class 6.2 requirements:

1. Infectious substances - contain or reasonably expected to contain pathogens, including bacteria, viruses, parasites, fungi, or recombinant microorganisms known to cause disease.

2. Genetically modified organisms and micro-organisms that meet the definition of infectious- genetically modified organisms known or suspected to be dangerous to humans, animals or the environment; genetically modified organisms capable of altering animals, plants or microbiological substances.

3. Biological products - products derived from living organisms such as vaccines and diagnostic products.

4. Diagnostic specimens - human or animal material including excreta, secreta, blood, tissue, tissue fluids being transported for diagnostic and investigative purposes.

5. Clinical waste and medical waste - wastes derived from the medical treatment of humans, animals, or from biological research, have a relatively low probability that infectious substances are present.

6. Infected animals- a live animal known or suspected to contain an infectious substance. Infected animals must not be transported unless the infectious substance cannot be consigned by other means.

Biological agents must be appropriately packaged and labelled for transfer between two locations, even if these locations are in the same institution, as demonstrated in Figure 15. Examples of appropriate shipping documents are available in Appendix 2.

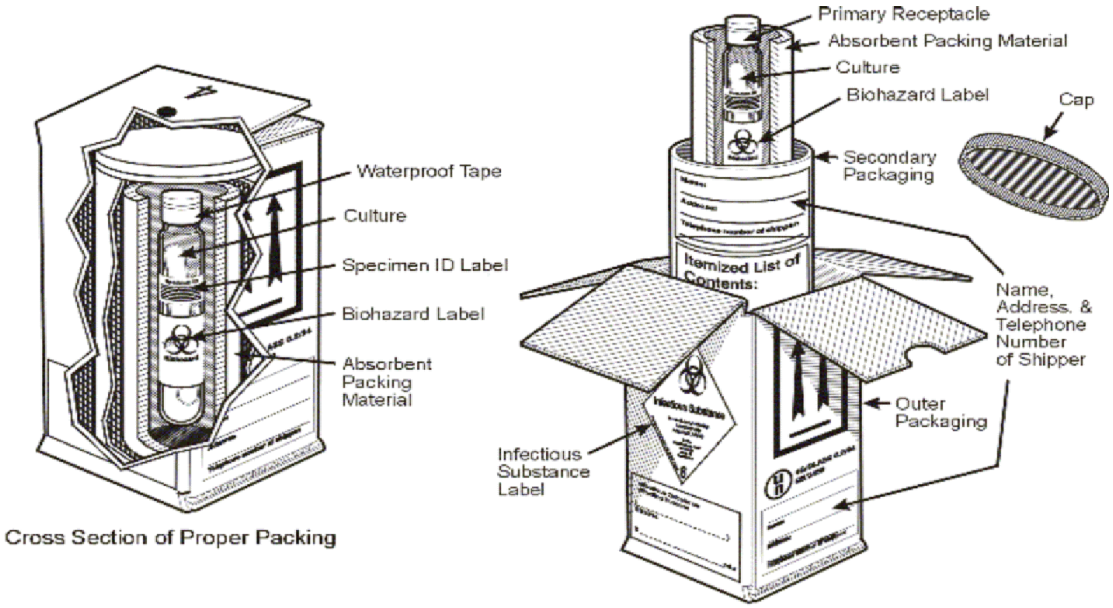


Figure 15. Proper packing and labeling of infectious substances.

Complete regulations can be reviewed by visiting the following web sites:

- Transportation of Dangerous Goods
- International Air Transport Association

Laboratories should also have approved procedures for the receiving and opening of packages that are marked with the biohazard symbol.

Appendix 1: Posters

Biological Spill Clean-Up Procedure

Biological Spill Clean-Up Procedure

Name and telephone number of the person responsible for enforcing safe work practices with biological agents in this work area:

Biological Safety Officer	Telephone #

Principal Investigator	Telephone #

Spill Within Biological Safety Cabinet (BSC)

1. ***Do not*** turn off the BSC.
2. Gather spill clean-up kit and don appropriate personal protective equipment (PPE).
3. Cover the area with absorbent material such as paper towel.
4. Apply an appropriate disinfectant beginning at the edge of the spill and working towards the centre. Pour disinfectant through perforated grills into a catch tray if spill has entered the grill.
5. Allow a minimum of 20 minutes contact time of the disinfectant before initiating actual clean up.
6. Use forceps to pick up any broken glass or sharps and place in a puncture-resistant container. Wipe up the spill from the outside towards the center and place materials into a biohazard bag (within the cabinet) for subsequent autoclaving.
7. All other materials within the cabinet at the time of the spill ***must*** be thoroughly cleaned and disinfected prior to removal from the cabinet.
8. Wipe down the inside surfaces of the cabinet with disinfectant and allow the BSC to run for at least 10 minutes prior to resuming work.
9. Remove PPE and place in autoclave bag for sterilization prior to laundering. Wash hands thoroughly.
10. Prepare an incident report for submission to the Biological Safety Officer.

Spill Outside the Biological Safety Cabinet (BSC)

1. Clear the area of all personnel.
2. If the spill has splattered the face, flush eyes and face with tepid water for a minimum of 15 minutes. Remove any contaminated clothing.
3. Cordon off the area with barricade tape to prevent others from entering.
4. Let the spill aerosols “settle” for at least 30 minutes before re-entering area to clean-up.
5. Don appropriate PPE (which at a minimum would include a gown, gloves and shoe covers). Depending on the nature of the spill an N-95 respirator may be required.
6. Cover the spill with an absorbent material working from the outside to the center of the spill.
7. Apply an appropriate disinfectant beginning at the edge and moving to the center of the spill. Allow 30 minutes of contact time.
8. After sufficient contact time, remove the absorbent material and any other items associated with the spill and transfer to a biohazard bag. Change gloves.
9. Spray the area with an appropriate disinfectant on the surface residue. Wipe and repeat this procedure once more.
10. Remove PPE and place in autoclave bag for sterilization prior to laundering. Wash hands thoroughly.
11. Prepare an incident report for submission to the Biological Safety Officer.

Working with Microbial Toxins

DALHOUSIE UNIVERSITY

Name and telephone number of the person responsible for enforcing safe work practices with microbial toxins in this work area.

Principal Investigator	Telephone #

Biological Safety Officer	Telephone #

Safety Practices For Working With Microbial Toxins

1. Prepare a Standard Operating Procedure (SOP) to cover procurement, storage, handling, decontamination, detoxification and disposal as well as a list of potential hazards associated with use, procedures for minimizing risk and training requirements.
2. Toxins shall be stored in locked facilities restricted to authorized personnel.
3. Wear appropriate PPE.
4. Gloves should be inspected and pressure tested for leaks prior to use if work involves the use of powdered toxins.
5. Preparation and manipulation of toxin stock solutions and primary containers of dry toxins as well as other high risk procedures must be carried out in a certified biological safety cabinet (BSC).
6. If a BSC is not available and dry powdered toxins must be manipulated in a chemical fume hood, face protection and an N-95 respirator is recommended. The fume hood exhaust must be HEPA filtered.
7. For high risk procedures (working with > 1 human lethal dose, intentional creation of aerosols, working with powdered/lyophilized toxins, creating primary containers and work with fast acting toxins) two knowledgeable individuals must be present.
8. Signs shall be posted at the laboratory entrance indicating that toxins are in use.
9. Before removal from containment, primary containers are to be decontaminated and placed in clean secondary containers.
10. Decontaminate the BSC regularly.
11. Deluge showers and eye wash stations must be readily available.

Decontamination

For most protein toxins, a sodium hypochlorite, or sodium hypochlorite and sodium hydroxide mixture provides effective decontamination. Surfaces may be decontaminated with a 0.5% solution of sodium hypochlorite. Solid and liquid waste may be decontaminated with a solution of 2.5% sodium hypochlorite and 0.25 N sodium hydroxide. They should be soaked or mixed in a 1:1 ratio and allowed to stand for 5 or 8 hours respectively.

Decontamination solutions should be tested routinely to ensure they are at the proper concentrations. When working with infectious organisms in conjunction with their toxins, care must be taken to ensure that both the infectious agent and the toxin have been neutralized.

BSL 2 Laboratory Rules

BSL 2 Laboratory

Biosafety Level 2 laboratories are suitable for work involving agents of moderate potential hazard to personnel and the environment.

24 hour emergency contact (name & phone number)

Required Signage

1. Dalhousie University Hazard Identification sign with a biohazard symbol and the Containment Level.
2. Label equipment housing the agent with the universal biohazard symbol.

Safe Work Practices

1. Keep laboratory locked when unattended.
2. Supervise biohazardous materials when in use.
3. Ensure that all staff have required training.
4. Do not consume or store food and/or drink in this laboratory.
5. Wear required personal protective equipment (PPE).
6. Mouth pipetting is prohibited.
7. Cover open wounds, cuts, scratches and grazes with waterproof dressing.
8. Maintain good housekeeping in the laboratory.
9. Limit the use of needles, syringes and other sharp objects.
10. BSCs must be used if the procedure produces aerosols, involves high concentrations or volumes of biohazardous material.
11. Maintain good personal hygiene (hand washing).
12. Work surfaces ***must*** be cleaned and decontaminated with a suitable disinfectant at the end of the procedure and after any spill.
13. Do not permit contaminated materials and equipment to leave the laboratory.
14. Participate in the biological indicator efficiency monitoring of your autoclave.

Storage and Waste Disposal

1. Refer to Dalhousie University's Policy for Management of Biological Waste..

Accidents and Spills of Biohazardous Material

1. Maintain a Biohazard Spill Kit (see the ***Biosafety Manual*** for a list of spill kit contents)
2. Follow spill clean-up procedures as outlined in the ***Biosafety Manual.***

Laboratory Requirements

1. Required personal protective equipment
2. Biohazard Spill Kit

Emergency Numbers

1. Security 9-902-494-4109
2. EH&S Office 9-902-494-2495

Autoclave Use

AUTOCLAVE USE

While operating the autoclave appropriate personal protective equipment ***must*** be worn, which consists of eye and face protection, heat resistant gloves and a lab coat. Each bag containing waste should include a sterilizer integrator.

24 hour emergency contact (name and phone number)

1. Packaging and Loading

- Use approved autoclave bags
- Prepare and load material to ensure steam penetration
- Do not seal bag
- Fill containers no more than 2/3 capacity
- Ensure sufficient water in load to allow steam penetration
- Use secondary containers
- Label all material (name, contact information)
- Ensure that material can be autoclaved (radioactive materials and some chemicals cannot be autoclaved)
- Do not mix clean and contaminated material in the same load
- Complete the autoclave use log
- Do not allow bags to touch the side of the autoclave or overload the autoclave.

2. Operating the Autoclave

- Ensure that the autoclave is operating properly
- Determine the appropriate exposure time
- Ensure that the autoclave attains the proper temperature, pressure and time desired
- Ensure that regular efficacy testing is performed on autoclave

3. Unloading the Autoclave

- Do not open until the chamber pressure gauge reads zero
- Open slowly to allow steam to escape
- Wait 10 minutes for the contents to cool
- Remove the material in such a manner that you reduce the risk of spillage
- Verify that temperature and duration of exposure have been met.

Biosafety Cabinet Use

Biosafety Cabinent Procedures and Operations

PROCEDURE	OPERATIONS
PLANNING	▪ Preplan procedure to ensure that you understand the procedure and that you have gathered all necessary equipment ▪ Ensure that room traffic will be kept to a minimum during your procedure to avoid creating air currents
START-UP	▪ Turn off the UV light* ▪ Set the sash in the correct operating position ▪ Turn on the fluorescent light and cabinet blower ▪ Ensure that return air grilles are not obstructed and note the pressure gauge reading ▪ Allow the cabinet to run 15 minutes before using ▪ Wash hands thoroughly before proceeding ▪ Wear required personal protective equipment (PPE) which would include a gown, over the cuff gloves, eye protection and mask if required **The use of UV light to decontaminate BSCs is discouraged*
CABINET PREP	▪ Wipe down the interior surfaces with 70% ethanol (or other suitable disinfectant) and allow to dry ▪ Place only necessary items for the procedure in the cabinet ▪ Do not obstruct the areas near the front or rear air grilles ▪ Do not position large items close together ▪ Wait 2 -3 minutes before beginning procedure to ensure that any airborne contaminants introduced during preparation have been purged
PROPER WORK PROCEDURES	▪ Work as far to the rear of the cabinet as is possible ▪ Segregate clean and contaminated materials working Left to Right, or Right to Left ▪ Arrange materials so as to minimize the movement of contaminated material into clean areas ▪ Do not hang spray bottles on the outside grille ▪ Avoid the excessive movement of materials, as well as operator's arms and hands, through the front access during your operation that will disrupt air flow ▪ ***The use of open flames is prohibited.*** ▪ If there is a spill or splatter during the operation, decontaminate items before removal. This must be done with the cabinet running.

POST PROCEDURE	■ After completion of your work, allow the cabinet to operate for 5 minutes to purge any airborne contaminants ■ Surface decontaminate items before removing from cabinet ■ Cover open trays or containers before removing from cabinet ■ Wipe down the interior surface of the cabinet with 70% ethanol (or suitable decontaminate) and allow to dry ■ Periodically remove the work surface and wipe down the underside of the work surface as well as the area underneath the work surface ■ remove first pair of gloves within the cabinet and place in the biohazard bag ■ Remove PPE ■ Turn off the fluorescent light and cabinet blower
ROUTINE MAINTENANCE	**WEEKLY** ■ Wipe down the entire interior surface of the cabinet with 70% ethanol or another suitable decontaminate ■ Using an appropriate glass cleaner clean the sash and the surface of the UV lamp (though the use of UV lamps is strongly discouraged) ■ Operating the cabinet blower, note the pressure gauge reading in the operational log **MONTHLY** ■ Using a damp cloth clean the exterior surfaces of the cabinet, particularly the top and front to remove any accumulated dust ■ Remove the work surface and thoroughly clean the undersurface as well as the area below the work surface with 70& ethanol or a suitable decontaminate ■ Check all service valves **ANNUALLY** ■ Have the cabinet recertified by a qualified certification technician

Appendix 2: Forms

A2.1 Record Keeping Forms

Biosafety Level 2 Laboratory – Visitor's Log

Section 31 of Bill C-11, Human Pathogens and Toxins Act, states –"A licence holder shall establish and maintain a list of all persons authorized by the licence holder to access the facility to which the licence applies, including persons holding a security clearance for that facility and visitors. The licence holder shall provide the Minister with that list if requested."

Date	Name	Affiliation	Time In	Time Out	Initials

Inventory

Biological Material or Agents Used/Stored

Biological Material/Agent	Host Ranges	Risk Group	In Use	Storage

Autoclave Use Log

Model#: ______________________ Series: __________________

Room#: __________________

Date	P.I	Description of Contents	Cycle selected	Temperature Achieved	Length of time autoclaved	Signature

Autoclave Efficacy Test Results

Make: ___________________ Model: ___________________ Serial #: _______________
Room #: __________________

Week of	Cycle Selected	Cycle Time	Spore Test *Indicate with a ✓*	Integrator Test *Indicate with a ✓*	Results Pass (P) Fail (F)	Signature

A2.2 Worker and Autoclave Registration Forms

Authorized Workers

To be designated authorized to work with biological materials/agents, the worker agrees to read Dalhousie University's Biosafety Manual and the *Canadian Biosafety Standards and Guidelines*.

Worker Name	Required Reading Completed

Autoclave Registration Form

Contact Information

Department	
Building	
Room #	
Contact Name	
Contact Phone	
Contact e-mail	

Autoclave Information

Manufacturer	
Model number	
Serial number	
Date of purchase	
Primary use	

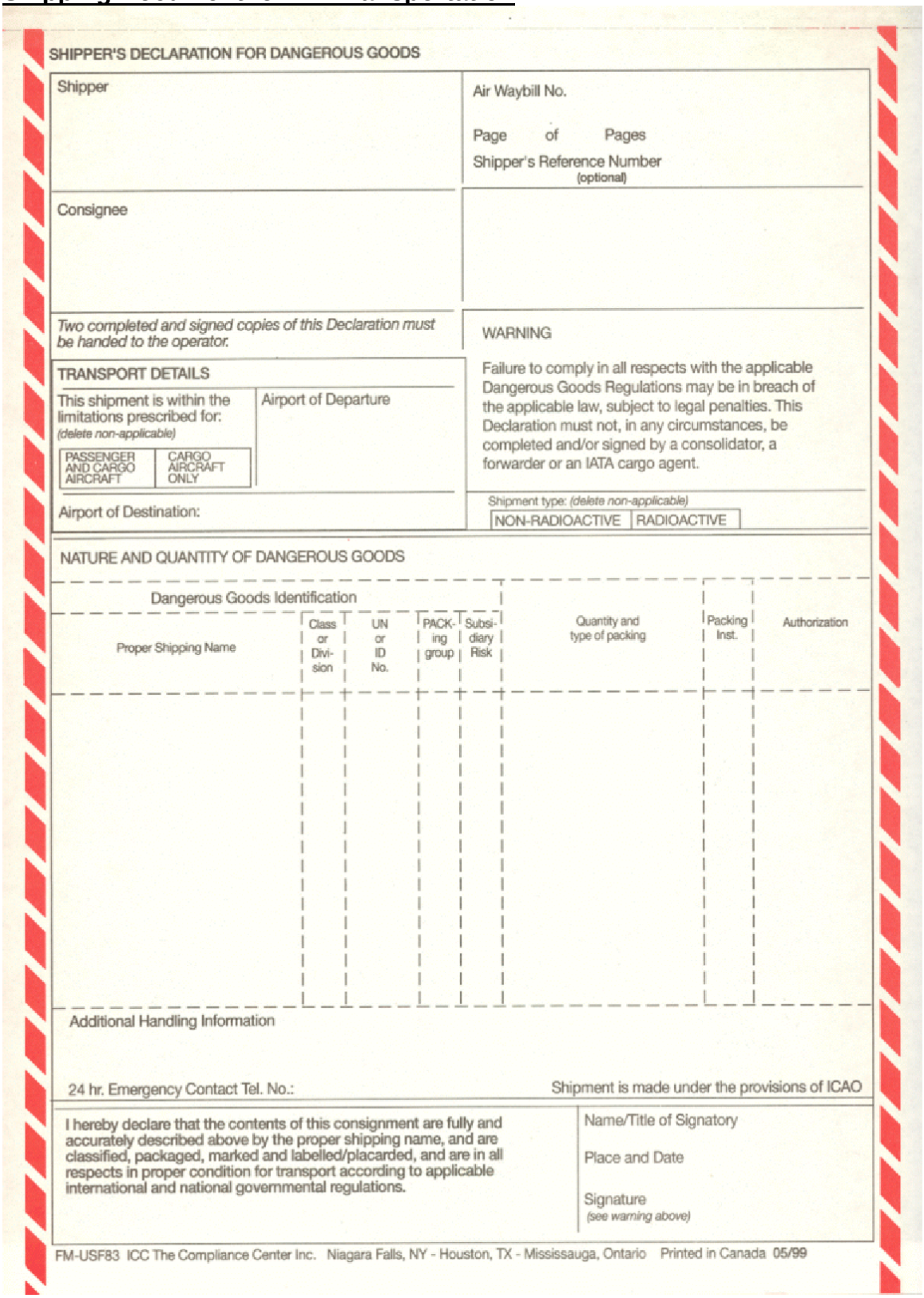
A2.3 Transport of Dangerous Goods Forms

Shipper's Declaration for Dangerous Goods, Shipped by Road

Consignor		**Consignee**
Carrier:		**Shipping Document #:**
Number of Packages	**Description of Articles**	**Weight or Volume of Package**
Special Handling:		
24 Hour Emergency Response Telephone Number: Dalhousie University Security - 902-494-4109		

Consignor's Declaration: "I hereby declare that the contents of this consignment are fully and accurately described by proper shipping name and are classified, packed, marked and labelled, and are in all respects in proper condition for transport by air and road according to the applicable international governmental regulations."		
Signature:	Date:	Phone Number:

Shipping Document for Air Transportation



SHIPPER'S DECLARATION FOR DANGEROUS GOODS

Shipper

Air Waybill No.

Page of Pages

Shipper's Reference Number (optional)

Consignee

Two completed and signed copies of this Declaration must be handed to the operator.

WARNING

Failure to comply in all respects with the applicable Dangerous Goods Regulations may be in breach of the applicable law, subject to legal penalties. This Declaration must not, in any circumstances, be completed and/or signed by a consolidator, a forwarder or an IATA cargo agent.

TRANSPORT DETAILS

This shipment is within the limitations prescribed for: *(delete non-applicable)*

PASSENGER AND CARGO AIRCRAFT | CARGO AIRCRAFT ONLY

Airport of Departure

Airport of Destination:

Shipment type: *(delete non-applicable)*

NON-RADIOACTIVE | RADIOACTIVE

NATURE AND QUANTITY OF DANGEROUS GOODS

Dangerous Goods Identification							
Proper Shipping Name	Class or Division	UN or ID No.	Packing group	Subsidiary Risk	Quantity and type of packing	Packing Inst.	Authorization

Additional Handling Information

24 hr. Emergency Contact Tel. No.:

Shipment is made under the provisions of ICAO

I hereby declare that the contents of this consignment are fully and accurately described above by the proper shipping name, and are classified, packaged, marked and labelled/placarded, and are in all respects in proper condition for transport according to applicable international and national governmental regulations.

Name/Title of Signatory

Place and Date

Signature *(see warning above)*

FM-USF83 ICC The Compliance Center Inc. Niagara Falls, NY - Houston, TX - Mississauga, Ontario Printed in Canada 05/99

Appendix 3: Biosafety Applications

Application for Biosafety Permit

An application for a Dalhousie University Biosafety Permit must be accompanied by a completed Dalhousie University Biohazards Risk Assessment Form, which can be obtained by contacting the Biosafety Office.

Principal Investigator	
Department	
Phone # :	
E-mail	

Work Location(s) and Containment Level(s)			
Building			
Room #'s			
Outdoor facilities if applicable			
Agent room storage location(s)			
Containment Level		□ Level 1	□ Level 2
Do you have a biosafety cabinet		□ Yes	□ No
Biosafety cabinet Model No.		Serial No. Certification Date:	
Is the work area a designated "red coded" lab ?		□ Yes	□ No
Location of autoclave for sterilization purposes:			

The applicant warrants the statements contained herein and agrees that the biological material or agent(s) used shall only be used in accordance with the information provided to the Dalhousie University Biosafety Officer in the ***"Dalhousie University Biohazards Assessment"*** form.

Signature of Applicant: _____ **Date:** _____

Signature of Department Head: _____ **Date:** _____

Application for the Renewal of Biosafety Certificate

With reference to Biosafety Certificate #:

Number : _____ **Expiring :** _____

Project Director: _____ **Department :** _____

Approval is requested for renewal of the above certificate (please check one)

_____ under the same conditions as the original application

_____ with changes as requested on attached application for amendment

An up to date list of workers who will use/handle biological agents (under your supervision) must be appended to this application. The list of workers will form part of the renewed certificate.

Date : _____ **Signature:** _____

Approved by : _____ **(Biosafety Committee)**

Date : _____

Form : BS - 001

Application for Biosafety Certificate Amendment

With reference to Biosafety Certificate #:

Number : _____ **Expiring :** _____

Project Director: _____ **Department :** _____

The following changes are proposed :

1. Addition of new agent (please specify) _____
2. Deletion of agent: _____
3. Change project director to: _____
4. Change location to: _____
5. Other (specify; new autoclave, containment cabinet, etc.,):
   _____
   _____

Date: _____ **Signature:** _____

Approved by: _____ **(Biosafety Committee)**

Date: _____

Form: BS- 002

Appendix 4: Biosafety Self Audit

DALHOUSIE UNIVERSITY

Biosafety Self-Audit Checklist

Principal Investigator: _____

Biosafety Certificate #: _____ **Location:** _____

Audited By: _____ **Date:** _____

Posting:

				Verification by Biological Safety Officer (Office Use Only)		
	Yes	**No**	**N/A**	**Yes**	**No**	**Comments**
Dalhousie University ***"Hazard Identification"*** poster with biohazard symbol posted on lab door						
Containment Level of the lab posted on lab door						
Emergency contact information posted						
Biohazard signs and/or symbols posted on storage and use areas						
Biohazard symbol posted to potentially contaminated equipment						
Biohazardous waste stored and treated appropriately						
Refrigerators labeled *"Not For Storage of Food for Human Consumption"*						
Dalhousie University ***Biosafety Manual*** available						
PHAC ***"Canadian Biosafety Standards and Guidelines"*** available (online acceptable)						

Facility:

				Verification by Biological Safety Officer (Office Use Only)		
	Yes	No	N/A	Yes	No	Comments
General housekeeping satisfactory						
No food or drink consumption permitted in the laboratory						
Containment cabinet operational and in good repair						
Containment cabinet certification current						
Designated clean areas present						
Hand washing facility available						
Eye wash station available						
Safety shower available						
Work surfaces cleanable and impervious						
Chair cover fabric readily decontaminated						
Autoclave working with calibration and log maintained						
Autoclave registered with EH&S						
Monthly biological indicator testing done on autoclave						
Centrifuge in good working order						
Aerosol producing equipment contained in devices that exhaust through HEPA filters						
Mechanical pipetting devices available						

Biosafety spill kit available						
Sharps containers available						
Laboratory doors are closed at all times						
Standard Operating Procedure (SOP) manual available and procedures followed						
A local risk assessment has been performed for procedures and processes that pose a risk, and an SOP has been generated to mitigate this risk						

Personnel:

				Verification by Biological Safety Officer (Office Use Only)		
	Yes	**No**	**N/A**	**Yes**	**No**	**Comments**
Lab coats worn						
Appropriate disposable gloves worn						
Eye protection available and worn						
Long hair tied back or restrained						
Suitable footwear with closed toes and heels worn by personnel						
Lab coats and gloves removed before exiting designated biohazard area						
Proper work procedures followed to avoid contamination and aerosol creation						
Mouth pipetting forbidden						
The use of needles, syringes and other sharp objects limited to avoid needle stick injury						
Personnel know and follow proper glove removal technique						
Personnel know and follow proper hand washing technique						

Workers are registered with the EH&S office, and posted approved worker list is up to date						
Workers have read the Dalhousie University ***Biosafety Manual***						
Workers been trained in proper use of containment cabinets						
Workers been trained in the safe use of autoclaves						
Workers been trained in effective decontamination techniques for agent used						

Biosecurity:

				Verification by Biological Safety Officer (Office Use Only)		
	Yes	**No**	**N/A**	**Yes**	**No**	**Comments**
Laboratory locked when room is unoccupied						
List of biological agents is current and has been approved by the Biosafety Office						
Biological agents secure against unauthorized removal						
Inventory records up-to-date to detect any loss or theft						
All keys issued are accounted for						
Access to the laboratory is limited to authorized personnel						

Disinfection, Decontamination & Waste Disposal

				Verification by Biological Safety Officer (Office Use Only)		
	Yes	**No**	**N/A**	**Yes**	**No**	**Comments**
Work surfaces decontaminated at least daily and immediately after a spill						
Decontamination techniques effective against agents used						
Waste disposal as per Dalhousie University's Policy for Management of Biological Waste, which is available for reference in the University's ***Biosafety Manual.***						

Emergency Response:

				Verification by Biological Safety Officer (Office Use Only)		
	Yes	**No**	**N/A**	**Yes**	**No**	**Comments**
Emergency spill procedures posted						
All personnel are aware of the requirement to immediately report accidents & spills that result in exposure to the P.I.						
All personnel are aware of the requirement to report accidents & incidents to the EH&S office, and know where to find the Incident Report Form						
During this past audit period, the P.I. has reviewed emergency response procedures with personnel						
During this past audit period, the P.I. has reviewed the location of spill kits, eye wash stations, and safety showers with personnel						

This laboratory is classified as:

Level 1 ☐ Level 2 ☐ Level 3 ☐

During this past audit period the following biological agents were used:

Bacterial	☐	rDNA	☐
Viral	☐	Toxins	☐
Fungal	☐	Animals	☐
Parasitic	☐	Human/primate blood	☐
Rickettsial	☐	Human body fluid, cells	☐
Prions	☐		

Other potential infectious material, specify _____

Office Use Only:

Verification:

Date: _____

Officer: _____

Signature: _____

Appendix 5: Biosafety Policies

Policy for the Security of Biological Agents

DALHOUSIE UNIVERSITY

Title:	Policy for the Security of Biological Agents
Number:	BSP - 01
Date:	July 31, 2009
Approved by:	Biosafety Committee

Introduction:

Ensuring the security of biological agents consists of two components:

a) **accountability**
b) **physical security**

Your accountability program has important security ramifications. You must maintain an accurate record of your inventory in order to know what is missing should loss or theft occur. Physical security means ensuring that a mechanism is in place either by a lockable laboratory door and/or a locked storage area to ensure that unauthorized removal of biological agents does not occur.

Section 4.1.11 of the *Canadian Biosafety Standards and Guidelines (CBSG)* outlines the requirements of a Biosecurity plan:

"A biosecurity plan, based on a biosecurity risk assessment, to be implemented, evaluated and improved as necessary, and kept up to date."

As per the CBSG, biosecurity plans commonly address items such as physical security, personnel management, infectious material accountability, inventory, incident and emergency response, and information security.

Physical Protection:

Biological Agents in use:

Constant surveillance and control must be maintained over biological agents in use. This means that an individual who has received training as approved by the Dalhousie

University Biosafety Committee, in the safe use of biological agents ***must be present*** in the laboratory during use.

Biological Agents in storage:

All biological agents in storage must be secured from unauthorized removal or access. The laboratory must be equipped with a lock. Certain biological agents as identified in The Australia Group's Human and Animal Pathogens and Toxins export control list as well as the CDC's Select Agents and Toxins list must be secured in a lockable storage facility within the laboratory when not in use.

When a room containing biological agents is unoccupied for periods such as lunch, meetings, after hours, etc. the ***room/and or storage enclosure must be locked***. Storage of biological agents in hallways is not permitted. Any exceptions to this policy must be approved by the Biosafety Committee.

Biohazard bags must be secure from unauthorized removal.

Personnel Security:

Every new worker must receive Biosafety training as approved by the Dalhousie University Biosafety Committee. Every new worker ***shall*** be informed of the Biosecurity plan.

Visitors to the laboratory as defined in the "Access to Facilities" policy will need to log in/out and must be under the supervision of an approved worker.

Background checks and security clearances will be required before workers are granted access to Level 3 facilities. Workers in Level 3 facilities will be required to have photo identification badges.

Pathogen Accountability:

Pathogen accountability procedures must include:

i) proper labeling
ii) tracking of internal possession
iii) inactivation and disposal of cultures after use
iv) transfers within and outside the facility

The inventory controls also assist in keeping track of agent storage locations and under whose responsibilities these agents lie. Inventories must be updated regularly. Record keeping should include not only the agent inventory, but also who accesses the agents as well as any transfer documents.

Biosecurity Incident and Incident Response:

The Principal Investigator or his/her designate must contact the Biological Safety Officer within 24 hours of any actual or suspected loss or theft of biological inventory. An investigation must begin immediately.

Responsibilities:

Responsible Officer (RO)

The responsible officer will typically be the Biological Safety Officer. The RO is responsible for:

i) Developing and implementing safety, security and emergency response plans.
ii) Ensuring that workers are adequately trained and are familiarized with the Biosecurity plan.
iii) Ensuring that any transfers of biological agents are done to comply with all regulatory requirements and that the agent is transferred to another licensed individual.
iv) Maintain detailed records of approved workers, inventory acquisitions, transfers, storage, and use locations.
v) Conduct regular inspections (as determined by the Biosafety Committee) of all facilities where biological agents are used and/or stored.

Responsibilities of Users:

i) It is the responsibility of the Principal Investigator to secure biological agents in storage from unauthorized access or removal.
ii) It is the responsibility of the Principal Investigator or his/her designate to maintain surveillance over biological agents that are not in storage.
iii) If constant surveillance cannot be maintained, biological agents must be secured.
iv) ***Biological agents must be stored in such a manner that an individual with authorized access to the area, but who is not authorized to use or possess the agents, cannot gain control of the agents.***
v) The Principal Investigator or his/her designate must contact the Biological Safety Officer within 24 hours of any actual or suspected loss or theft of a biological agent.

Access to Facilities

DALHOUSIE UNIVERSITY

Title:	Access to Facilities
Number:	BSP - 02
Date:	July 31, 2009
Approved by:	Biosafety Committee

The "**Human Pathogens & Toxins Act"** states:

31. "A licence holder shall establish and maintain a list of all persons authorized by the licence holder to access the facility to which the licence applies, including persons holding a security clearance for that facility and visitors. The licence holder shall provide the Minister with that list if requested to do so."

Level 2 Facilities:

1. Security clearance is NOT required for facilities handling and/or storing Containment Level 2 biological agents.
2. Biosafety Certificates will include a list of trained workers authorized to handle biological agents.
3. Biological toxins and biological agents appearing on The Australia Group list and the Centers for Disease Control and Prevention Select Agent list shall be locked up when unattended by a person appearing on the Biosafety Certificate approved worker list.
4. Faculty members, holding a Dalhousie University Biosafety Certificate, shall maintain a class list of all students who will have occasion to meet with the faculty member in his/her office within the laboratory.
5. Dalhousie University (Human Resources), on behalf of the faculty members holding a Dalhousie University Biosafety Certificate, shall maintain a list of all Dalhousie staff who will have occasion to enter a facility handling/storing Containment Level 2 biological agents.
6. A log book shall be maintained by each lab holding a Biosafety certificate to list visitors, not covered in any of the points above such as external contractors, sales representatives, etc. The list of visitors in these log books shall be maintained for three years. Authorization from the Biological Safety Officer is required to dispose of these records.
7. Visitors to Containment Level 2 facilities will be required to abide by Dalhousie University's *"BSL 2 Laboratory"* rules.
8. The Biosafety Committee has the authority to decline access to a Level 2 facility.

Policy for Management of Biological Wastes

DALHOUSIE UNIVERSITY

Title:	Policy for Management of Biological Waste
Number:	BSP - 03
Date:	January 19, 2009
Approved by:	Biosafety Committee

Introduction:

Dalhousie University generates types of hazardous waste including biohazardous waste. Managing the waste stream is ***everyone's responsibility.*** Transportation and disposal of biohazardous waste is subject to provincial and federal regulations. Failure to observe appropriate disposal practices can cause harm to people, animals and the environment. Failure to follow appropriate practices can also lead to prosecution.

It is a basic biosafety principle that all contaminated materials be decontaminated prior to disposal. Decontamination includes both sterilization (the complete destruction of all microorganisms, including bacterial spores) and disinfection (the destruction and removal of specific microorganisms).

Categories of Biological Waste:

Solid Waste:

Some examples of solid biological waste (but not limited to) may include:

i) Gloves and other disposable PPE that is contaminated
ii) Pipettes, pipette tips, culture plates, specimen vials that have been contaminated with biological specimens, bacterial and cell culture material, or nucleic acids
iii) Bench or tray liners used in areas where biological agents are manipulated
iv) Disposable primary containers with < 10 ml. of liquids

Liquid Waste:

This includes quantities > 10 ml of blood, blood products, body fluids (human or animal) and culture media.

Sharps:

A sharp is any device that is sharp enough to puncture the skin and that is contaminated with a biological agent that is an infectious disease transmission risk, or an environmental risk.

Some examples of sharps include:

i) Needles, disposable syringes, capillary tubes, scalpels
ii) Microscope slides contaminated with unfixed human or animal specimen materials
iii) Pipettes contaminated with cell culture waste media
iv) Broken tubes of blood or culture

Disposal and Packaging Procedures:

Autoclaving potentially infectious waste is the preferred method of rendering biological waste non-infectious prior to disposal. However, waste containing materials such as ***phenol or formaldehyde*** must not be autoclaved but rather rendered non-infectious by chemical methods.

Autoclaving:

i) Material and/or items potentially contaminated with a biological agent must be placed in a clear autoclave bag with no markings.
ii) When sufficiently full the bag (no more than 2/3 full) should be placed in a second autoclave bag to ensure no secondary contamination should the outside of the original bag be contaminated.
iii) Bags should be ***loosely*** closed and placed in a covered waste bin until the material can be autoclaved.
iv) Before autoclaving ensure that the bag is ***open*** to allow steam penetration.
v) A sterilizer integrator strip must be added to each load to ensure proper sterilization has taken place.
vi) Bags must be held prior to final disposal until results from biological indicator testing have been received.
vii) Bags must bear an approved label indicating: date, lab of origin, and a telephone number
viii) The autoclaved waste must be transported to the local facility used to hold animal tissue and related wastes pending collection for final disposal.

Chemical Treatment of Tissue and Microbiological Wastes:

i) In a ventilated enclosure such as a fume hood, submerge tissue culture or microbiological waste in a solution prepared by diluting one volume of household bleach with 10 volumes of water.
ii) Allow a minimum of 12 hours contact time.
iii) Following treatment, decant the bleach solution and flush it down the drain with large volumes of water. Package the chemically treated waste in a secure container. Container must bear an approved label indicating: date, lab of origin, and a telephone number
iv) Transport to the local facility used to hold animal tissue and related wastes pending collection for final disposal.

Disposal of Wastes Containing Animal Tissue

All animal tissue and related waste must be double bagged in polyethylene bags. At least the outer bag must be a standard ***orange*** coloured bag and enable waste handlers to identify the contents

i) Package weight must not exceed 22 kilograms**.** For packages exceeding this weight contact the Manager of Environmental Services at 902-494-6779.
ii) All packages containing animal tissue and waste must be securely taped shut and labelled with the name and telephone number of the laboratory supervisor. Species of animal must also be included.
iii) While awaiting disposal, labelled and secured packages of animal tissue and waste must be properly stored in one of the storage facilities listed above.
iv) Animal tissue and waste containing radioactivity, significant quantities of toxic chemicals or infectious organisms must be disposed of following the advice of the University Veterinarian**,** the Director of the Carleton Animal Care Facility and/or the Radiation Safety Officer.

Disposal of Research Sharps

Research in science and medicine often involves the use of needles, scalpels and similar sharp items. In some applications, this equipment becomes contaminated with chemical, radiochemical or infectious material which presents a disposal problem. Even if uncontaminated, inappropriately discarded sharps could injure people who handle refuse.

To avoid injury to those who handle University waste and to prevent environmental damage, all research sharps ***shall*** be disposed of as set out in the following procedure.

Sharps may ***NOT*** be disposed of along with normal building refuse.

Sharps may ***NOT*** be mixed with waste scintillation fluid.

i) All used needles, scalpel blades and other sharp items that are discarded should be placed in labelled commercial sharps disposal containers
ii) When the container is three quarters full, close with the attached lid and seal with tape. Indicate the name and telephone number of the laboratory supervisor on the label. Also note any unusual chemical, radiochemical or infectious hazard.
iii) Used sharps will be collected for disposal at the same time as waste solvents.

Disposal of Dental Clinic Solid Waste

Providing dental care creates wastes which must be handled safely and in accord with local, provincial and federal regulations as set out in the following procedure:

Used disposable items (rubber dams, cotton rolls, gauze, suction tips etc.,) are to be placed in a plastic bag, tied closed and placed in the cubicle waste container.

i) Clinic waste (gauze) that is saturated with blood will be immersed in a solution (1 oz. to 1 gallon of water) of Metriclean, until the blood is dissolved.
ii) Liquid waste will be poured into the sanitary sewer drain. Once excess liquid is removed, gauze will be placed in a plastic bag and tie closed.
iii) Regardless of its origin, ***no*** fluid may be disposed of with normal clinic waste.
iv) Sharps may not be mixed with clinic solid waste.
v) Corrosive or flammable fluids or those that contain toxic components may not be poured into the sanitary sewer drain. For advice contact the Safety Office at 902-494-2495.

Disposal of Dental Clinic Fluid Wastes

Providing dental care creates wastes which must be handled safely and in accord with local, provincial and federal regulations as set out in the following procedures

i) Body fluid should be poured into the sanitary sewer drain.
ii) Glass collection bottles used at off-site clinics should be cleaned and dried before being re-attached to the suction system.
iii) Plastic collection bottles and suction tubing used in Oral Surgery should be rinsed free of blood in Room 1466 prior to being placed in the general clinic waste.
iv) Prepare ***Presept*** disinfectant and run it through suction tubing into a collection device. Let stand for three minutes before pouring into a sanitary sewer drain.
v) Corrosive or flammable fluids or those that contain toxic components may not be poured into the sanitary sewer drain. Contact the Safety Office at 902-494-2495 for advice.

Disposal of Dental Clinic Sharp

Used needles, scalpel blades and other sharps are to be placed in puncture resistant containers at the site of use.

When the container is three quarters full, seal the container with the attached lid and transport to Room 2500A for storage prior to disposal by incineration.

Compliance Enforcement Policy

DALHOUSIE UNIVERSITY

Title:	Compliance Enforcement Policy
Number:	BSP-04
Date:	September 10, 2010
Approved by:	Biosafety Committee

Introduction:

The Dalhousie University Biosafety program is based on the requirements of the Public Health Agency of Canada's "Pathogen Regulation Directorate" and the Canadian Food Inspection Agency.

In June of 2009 the Parliament enacted into law the "Human Pathogens and Toxins Act". In the near future the university will be issued a license covering all regulatory aspects of the use of biological agents that may cause disease in humans.

The Biological Safety Officer, who is responsible for the day to day operations of the Biosafety program, reports to the Biosafety Committee which has the authority to implement and enforce the Biosafety program encompassing the use, handling, storage and safe disposal of biological agents. The Biosafety Committee is appointed by and accountable to the Vice-President, Finance.

The university may be visited by inspectors from both the Canadian Food Inspection Agency and the Public Health Agency of Canada. Under the Human Pathogens and Toxins Act, the Public Health Agency of Canada has the ultimate authority to withdraw user privileges if serious violations are observed. A serious violation could result in monetary fines and/or incarceration and may also impact all others using biological agents at the university.

Compliance Audit Policy:

Each research group will be required to conduct a Biosafety Checklist audit twice yearly, in May and November. These audits will be reviewed by the Biological Safety Officer and any corrective action required must be completed within one week of notification.

The Biological Safety Officer will endeavor to visit laboratories to which a Biosafety Certificate is issued at a rate of one per month. The visit will be unannounced. Selection criteria for the visit may include:

- Major changes to the Biosafety Certificate
- Significant changes to the approved worker list
- The occurrence of an accident or incident

- Timely submission of self-audits
- Concerns raised by lab personnel or others
- Time period since last inspection by Biological Safety Officer

A compliance checklist approved by the Biosafety Committee will be used for inspections by the Biological Safety Officer. Violations will be categorized as either major or minor offences.

A **major offence** would result from violations which cause immediate risk or danger to safety, health, loss or theft and negligent release to the environment. Examples of a major offence would include:

a) consumption or storage of food or drink in the laboratory
b) inadequate training of staff
c) refusal to wear required personal protective equipment (PPE)
d) inadequate security measure to safeguard biological agents
e) failure to report loss or theft of a biological agent
f) negligent release of a biological agent to the environment
g) failure to report laboratory required infections (LAI's)
h) failure to have biosafety cabinet recertified within a twelve month period.

A **minor offence** would be an infraction which poses no immediate risk or threat to health, safety or the environment. Examples of a minor offence would include:

a) inadequate signage
b) inadequate posting (BSL 2 poster, Spill clean-up procedure)
c) inadequate inventory records
d) inappropriate use of warning labels
e) failure to document transfers

MAJOR OFFENCE ACTIONS

1. On the **first** occurrence, written notification will be sent to the permit holder, with a copy to the department head, outlining the nature of the offence. Immediate attention to and correction of the violation is required.

2. On a **second** occurrence within a twelve month period the permit holder will be notified in writing that the permit will be revoked until a meeting can be held with the Biosafety Committee. The certificate holder may attend the meeting to explain why his/her permit should be renewed.

3. On a **third** occurrence within a twelve month period the permit will be cancelled and all inventory disposed of by the Biosafety Office.

   For the second or third occurrences notification of the above actions will be copied to the department head and the Dean.

MINOR OFFENCE ACTIONS

1. On the **first** occurrence, the permit holder will be notified verbally by the Biological Safety Officer of the violation observed.

2. On a **second** occurrence within a twelve month period the Biological Safety Officer will send written notification of the observed violation to the certificate holder, with copies to the department head and the Biosafety Committee.

3. On a **third** occurrence, within a twelve month period the Biological Safety Officer will arrange to have the certificate transferred to the Head of the department in which the certificate holder does the majority of his/her work. If the department head agrees to assume responsibility all work will be under his/her direct control. The department head's signature **must** appear on all purchase requisitions. Written notification of the above action will be sent to the Dean of the faculty.

4. On a **fourth** occurrence within a twelve month period the certificate will be revoked. A meeting may be requested by the permit holder with the Biosafety Committee at which time the certificate holder may argue as to why the permit should be renewed.

Minor offences must be corrected within seven (7) calendar days.

Termination of Use of Biological Agents

DALHOUSIE UNIVERSITY

Title:	Termination of Use of Biological Agents – Renovations, Remodels, Moves, Terminations
Number:	BSP - 06
Date:	September 10, 2010
Approved by:	Biosafety Committee

Introduction:

A Principal Investigator (PI) is the individual in whose name the Biosafety Certificate is issued. ***The Principal Investigator is responsible to the university for the safe use of such agents by all persons under their supervision. Further, the Principal Investigator is responsible for the security of these agents from the time they enter the laboratory until they are safely and properly disposed of.***

It is the responsibility of the Principal Investigator that the Biological Safety Officer receives advance notification when:

1. there is a planned move to new laboratory space
2. there is expansion of current laboratory space (renovation)
3. there are changes to existing laboratory space (renovation/remodel)
4. work with biological agents ceases
5. the Principal Investigator leaves the university

Procedures:

1. Notify the Biological Safety Officer prior to any of the above listed changes or moves, giving the following information – name of PI, department, phone number, time and date of projected change or move.
2. Dispose of all biological waste as was done routinely for that location – through autoclaving, chemical disinfection etc. Follow Dalhousie University's "Policy for Management of Biological Waste" guidelines.
3. Render recombinant organisms inactive prior to disposal.
4. Render microbial toxins inactive following procedures outlined in Dalhousie University's ***"Working with Microbial Toxins".***
5. Clean and decontaminate work surfaces with a suitable disinfectant.
6. Empty biological safety cabinets, incubators, freezers, etc. and decontaminate with a suitable disinfectant.

7. Dispose of all sharps as per university policies as defined in “Policy for Management of Biological Waste”.
8. Deface hazard signage on equipment that has been decontaminated and remove door signs.
9. Biological agents not designated as waste must be disposed of in one of the following ways:
    a) an inventory ***transfer within the same department***
    b) and inventory ***transfer within the university***
    c) an inventory ***transfer to another institution.*** In case of such a transfer only individuals with class 6.2 TDG training will be permitted to ship. Documentation from the receiving institution must be forwarded to the Biological Safety Officer verifying that the receiving institution is approved to receive the material and that the institutional BSO approves the transfer.

The procedures listed above have been completed. The laboratory has been decommissioned and/or prepared for vacating/renovation.

_____ _____

Principal Investigator Date

_____ _____

Biosafety Officer Date

Transfer/Shipment of Biological Agents

DALHOUSIE UNIVERSITY

Title:	Transfer/Shipment of Biological Agents
Number:	BSP - 07
Date:	August 2010
Approved by:	Biosafety Committee

Transfers of biological agents are permitted. Transfers from one certificate holder to another within the same department are permitted by making appropriate notations on respective inventories, and providing that the receiver is authorized to use the particular agent being transferred. A transfer form is not required. Transfers from one certificate holder to another in a different department or a different physical location on campus are permitted providing the receiver is authorized to receive the particular agent being transferred. A transfer form is required as well as appropriate notations on respective inventories.

Transfers of biological agents that do not fall into the above category but will remain on campus must be coordinated by the Biological Safety Officer.

Transfers of biological agents to another institution must be coordinated through the Biological Safety Officer. The Biological Safety Officer will determine through contact with the Biosafety Officer at the receiving institution whether the shipment can be transported to and received. Coordination of the shipment must be done by an individual holding a valid TDG Class 6.2 certificate.

Prior to any transfer of biological agents to Dalhousie University from another institution approval must be granted by Dalhousie University's Biological Safety Officer.